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Successful Experiences and Good Practices in Chemistry Education

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TEACHING CHEMISTRY OF NATURAL PRODUCTS TO YOUNG STUDENTS: "*VERÃO CIÊNCIA NO IPB*" CASE STUDY

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Abstract

For some years the BioChemCore research group of School of Agriculture, Polytechnic Institute of Bragança (IPB), has conducted several one-week internships for young students in college, inserted in the program "Verão Ciência no IPB" (science summer in the IPB). These small courses aim to disseminate various aspects of the research conducted by our group, which include chemistry of natural products, nutraceuticals and functional foods, technology of natural products, and medicinal chemistry. In this case study, brief information was provided on all these topics, but highlighting the chemistry of natural products. This manuscript reports the assays, theory and goals achieved by the students enrolled in the internship, covering various aspects of chemistry and biochemistry, antioxidant activity, and finally the research on some bioactive molecules through high performance liquid chromatography. Although the receptiveness and motivation of students was high the details of the explanations were basic and (adjusted to their level of understanding) easy to comprehend. This yearly internship has been very successful over the various editions of the program, with an increasing number of students applying for it.

1. Introduction

In the last decades, chemistry of natural products has gained an increasing recognition, not only from the academic community, but also companies and general public. This phenomenon is mainly due to the recent discoveries regarding the potential of natural matrices in many fields, namely health, functional food and cosmetics. Many natural compounds and extracts have been increasingly present in our daily lives, either by addition to foodstuffs, inclusion in pharmaceutical and cosmetic preparations, as well as in polymers, plastics and other everyday use objects.

These natural products are interesting for their various bioactivities, displayed at very low dosages. Antitumor, antimicrobial and antioxidant potential are three of the most important bioactivities. These properties are possible due to a large diversity of bioactive molecules, like phenolic compounds and vitamins, among others. The population has become more aware and interested in the benefits of these compounds, and the need to inform younger audiences is increasing. So, how can chemistry of natural products be taught to young students?

Since 2008, the IPB organizes the "Verão Ciência no IPB" for young students, attending the 10th, 11th and 12th grade of high school. With this project, one-week internships are conducted in various laboratories, to show these students the research carried out in the institution. During the seven days, the students are introduced to the theory of the research topics they have chosen, and then tutored by researchers and graduate students while conducting experiments and assays in the laboratory.

Our research group, BioChemCore (www.esa.ipb.pt/biochemcore), led by Dr. Isabel C.F.R. Ferreira, is intrinsically linked to the study of chemistry of natural products, identifying and quantifying chemical compounds from various matrices, namely plants, mushrooms and dry fruits. Evaluation of antioxidant, antimicrobial and antitumor (human tumor cell lines) properties is also carried out by chemical and biochemical assays.

The internships focus on easy, guided, straightforward and hands-on assays. Initially safety measures, general knowledge of the laboratory and a theoretical introduction regarding chemistry of natural products is provided to the students. They are then divided in groups and all go through the various assays. Coverage of extraction methods, spectrophotometry, separation techniques, identification of biomolecules and some brief notions of chromatography are lectured. The broadness of the chemistry of natural products is summarized in Figure 1.

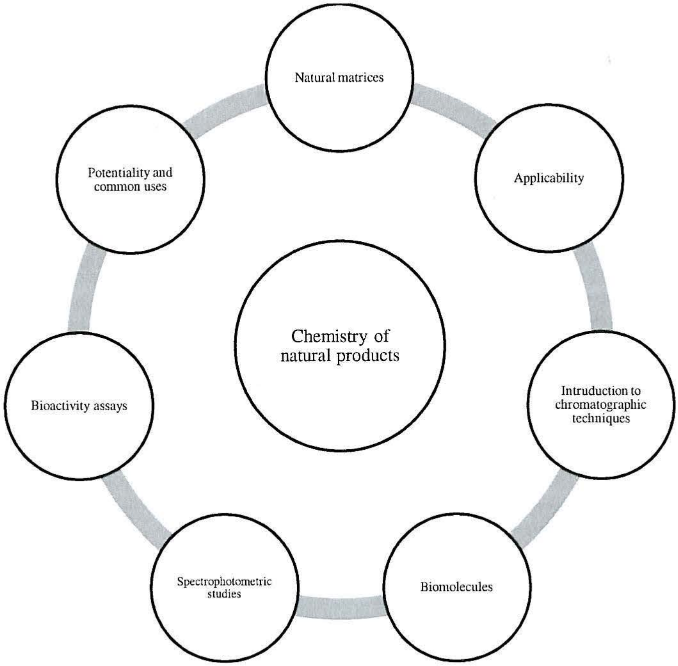

Fig.1. Steps used to introduce the students to chemistry of natural products.

2. Applied chemistry made easy

The antioxidant activities shown to the students cover various types of oxidation inhibition; free radicals scavenging activity, reducing power and lipid peroxidation inhibition.

2.1. Extraction

The extraction step is the first to be introduced. Typically, the student weigh the mass of extracts and measure the solvents selected for extraction. Then, while the extraction is taking place, theoretical concepts of simple extractions (solid-liquid, liquid-liquid) are mentioned. After evaporation of solvents, and determination of the solid residue, simple chemistry mathematical operations are solved to determine the ideal volume for a specific concentration. The various bioassays are then conducted in the various dilutions from the stock solution.

2.2. Antioxidant activity

Among the various antioxidant activity assays, the students are introduced to only four, covering antioxidant activity through chemical and biochemical assays (Figure 2).

Fig.2. Students performing antioxidant activity assays.

2.2.1. DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is based on the assumption that a hydrogen donor is an antioxidant, and therefore, the DPPH radical, by scavenging a hydrogen atom from surrounding molecules stabilizes itself and changes its color from deep purple to yellow with increasing antioxidant concentration.

In this experiment, students are proposed to use microplates to pipette DPPH over the antioxidant extract previously prepared (section 2.1). The most antioxidant concentrations of the extracts shift immediately to yellow while the intermediate ones tend to take longer due to antioxidants with lengthier reactions (i.e., polyphenols). After one hour the absorbance is read at 515 nm. The importance of successive dilutions to determine the IC_{50} is then explained to them [1].

2.2.2. Reducing power assay

This colorimetric assay can show the reducing power of extracts, by reducing Fe^{3+} to Fe^{2+}, which is accompanied by a color change from yellow to green, easily detected at 690 nm [2].

Students are briefly introduced to some notions of reduction reactions while doing the assay.

2.2.3. β-carotene bleaching inhibition

β-carotene is a natural pigment, which displays an orange color and is quite sensitive to oxidation. In this biochemical assay, linoleic acid is transformed into the linoleate radical, which then attacks the β-carotene structure. If this attack is successful, the pigment will lose its orange color and turn transparent.

The kinetics of the underlying reaction is briefly explained to the students, and finally the absorbance is spectrophotometrically determined at 470nm [3].

2.2.4. Thiobarbituric acid reactive substances (TBARS) inhibition

The final and most complex assay is left for last in order to allow the students to gain experience from the previous ones. This assay is based on the inhibition of reactive substances formed by peroxidation of porcine brain cells by the ascorbate radical. The complex formed between the malondialdehyde from the peroxidation and the thiobarbituric acid is then heated up to produce an

array of colors which vary according to the quantities of antioxidants present in the extracts. The final step is determining the absorbance at 532 nm.
The reactions taking place in the test tube are briefly explained to the students, mostly regarding peroxidation of lipids [2].

3. Bioactive molecules

When it comes to chemistry of natural products, it is important to mention specific biomolecules that provide important features to natural extracts. Phenolics (including flavonoids) and two different vitamins (tocopherols- vitamin E and ascorbic acid- vitamin C) are studied at the "Verão Ciência no IPB".

3.1. Total phenols

Total phenols are determined through the Folin-Ciocalteu assay, which is a mixture of molybdenum and tungsten and has a yellow coloration. Phenols or polyphenols are secondary metabolites produced by plants to protect themselves against predators, to promote growth and development of structures. Under alkaline conditions the polyphenols present in the solution are reduced by the molybdenum and tungsten, shifting the overall color of the solution from yellow to blue, depending on the quantity of reducing species in the solution. Although being used quite commonly, this method suffers from great interferences from compounds other than phenols that also reduce themselves, leading to overexpression of results. It should be considered an exploratory or preliminary method, to determine if there are reducing species in the mixture.
This concern and the basic of oxidation-reduction reactions are explained, helped by the visually appealing change in color of the solution during heating [3].

3.2. Total flavonoids

One of the most bioactive sub-group of polyphenols are the flavonoids. These compounds are characterized by three aromatic rings and fifteen carbons. When in contact with aluminium chloride in an alkaline mixture, these compounds form a complex that displays a pink color. Interference is minimized by the poor absorbance of other sub-groups at the ideal absorbance for flavonoids, 610 nm. Still, some researchers point out that not all classes of flavonoids react in the same way to aluminium chloride, therefore not contributing to the pink color, classifying this test as a preliminary assay. The kinetics of this assay as also its limitations are briefly explained to the students [4].

3.3. Ascorbic acid

Vitamin C, also known as ascorbic acid, has an important biological function in humans. However, it is not synthetized by them. This enzyme is an important cofactor of several enzymes and it is considered one of the most powerful antioxidants, with the capacity to scavenge electrons in a wide variety of chemical reactions.
The protocol used in the internship is the 2,6-dichloroindophenol assay, which relies on the capacity of vitamin C to reduce this compound, which turns from blue to pink instantly if the vitamin is present in the solution. The absorbance is determined at 515 nm. The visual attractiveness is quite high for this assay (Figure 3), and students are once again elucidated regarding the importance of this compound and its ability to neutralize radicals in living organisms [5].

Fig.3. Student preparing the test tubes for ascorbic acid determination.

3.4. Tocopherols

Tocopherols (included in vitamin E) comprise four different isoforms, α-, β-, γ- and λ-tocopherol. These molecules are essential components of the biological membranes of photosynthetic organisms. α-Tocopherol is of extreme importance to human nutrition, being able to act as an antioxidant, directly repairing reactive oxygen species, and preventing the propagation of lipid peroxidation.

The extraction protocol is quite complex due to the fact that tocopherols are degraded by heat, oxygen and light. After the extraction, tocopherols are submitted to extensive preparation for determination in a high performance liquid chromatography (HPLC) coupled to a florescence detector. This detector is chosen for the natural capacity of tocopherols to emit fluorescence. Tocopherols were left for the final lecture because their determination is made by HPLC, not directly handled by the students, but by staff of the research group. Very brief concepts about chromatography are provided and the results obtained are discussed (Figure 4) [6].

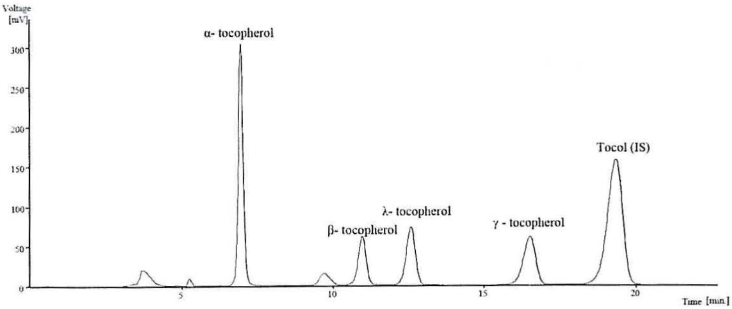

Fig.4. Chromatogram displaying the 4 isoforms of tocopherols and the internal standard (tocol).

4. Conclusions

Teaching chemistry of natural products to young students is a task that requires constant guidance due to the complexity of the subject, however the feedback in all the editions of the internships was quite positive. At the end of the internship it was expected that the students were able to:

- Know the various natural matrices studied in our research group;
- Carry out studies of bioactivity, including antioxidant properties;
- Know some bioactive molecules, such as polyphenols, vitamin C and E;
- Understand the theoretical and practical concepts of the experiments performed;
- Understand the basic concepts related to chromatography;
- Understand the future applications of the studies;
- Conduct scientific experiments in an expeditious manner.

With no final examination, the objectives were not quantified, although qualitatively the students saw their expectations fulfilled. Inquired after the internship, students felt they learned the basic concepts of chemistry of natural products as well as all linked themes related to the practical work done in this laboratory. The success of this internship has been considerable and an increasing number of students apply for it in every year. Although being quite ahead of their schooling, the studied examples are important to provide interest and motivation for the students to visualize practical applications of the chemistry they learn. Another important goal for this internship is to bring more interest to the broad subject of chemistry.

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