

APLICAÇÃO DA METODOLOGIA APRENDIZAGEM BASEADA EM PROJETOS (ABP) NA DISCIPLINA QUÍMICA ORGÂNICA POR MEIO DO ESTUDO DE MÉIS

APPLYING PROJECT-BASED LEARNING (PBL) IN THE ORGANIC CHEMISTRY COURSE WHILE STUDYING HONEY

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RESUMO: ABP sugere processo de ensino e aprendizagem centrado no estudante. Aplicou-se o projeto “Caracterização de mel pelo perfil em fenólicos e avaliação antioxidante, focando ABP” (dois méis florais finlandeses, um português, e um mel de melato finlandês) na “Química Orgânica” na 2ª série do Técnico em Química Integrado ao Ensino Médio no Instituto Federal Sudeste de Minas Gerais - Campus Barbacena. Trabalhou-se o conteúdo durante o andamento do projeto. Os grupos foram sorteados, buscando trabalho em equipe. Dialogicamente, valorizou-se a individualidade. No primeiro bimestre realizaram-se análises espectrofotométricas no ultravioleta-UV. Cada grupo deu aula para outro grupo, caracterizando o aluno como orientador. Eles foram os próprios autores do processo de aprendizagem em cooperação, relacionando mel com compostos orgânicos. Estudaram átomo de carbono, e iniciaram funções. Modelos moleculares visaram o conhecimento discutido (ligações simples/dupla/tripla, σ/π ; hibridação/geometria; fórmula estrutural). No segundo bimestre houve preparo de extrato por cromatografia, levando também ao conhecimento sobre propriedades dos compostos (ocorreria no quarto bimestre), aplicando o subprojeto “Séries Homólogas”. Novos grupos foram sorteados (3º bimestre), em uma aula dialógica motivadora. A confiança foi criada, do aluno no aluno, no processo de aprendizagem e nos professores. No último bimestre, pesquisaram resultados químicos preliminares. Os perfis fenólicos foram similares, com mais diferenças na amostra de melato finlandesa e floral portuguesa. Estudaram isômeros e completaram-se funções. Ocorreram avaliações diferenciadas para a construção de conhecimento, incluindo o uso de ferramentas tecnológicas. Houve surpreendente nível de motivação entre os participantes. A implementação de abordagem ABP centrada no estudante é eficaz e poderosa.

PALAVRAS-CHAVE: Aprendizagem Baseada em Projetos (ABP). Abordagem centrada no estudante. Aprender fazendo. Trabalho colaborativo. Química orgânica.

ABSTRACT: *PBL suggests that the teaching and learning process is student-centered. The project "Characterization of honey by phenolic profile and antioxidant evaluation, focusing on PBL" (two Finnish floral honeys, one Portuguese, and one Finnish honeydew) was applied in the "Organic Chemistry", to 2nd year students of Vocational Course in*

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Chemistry at the Federal Institute of the Southeast of Minas Gerais - Barbacena Campus. The content was worked throughout the project. The groups were mixed, seeking teamwork. Dialogically, individuality was valued. In the first period UV-spectrophotometric analysis were performed. The groups taught each other, characterizing the role of the student as an advisor. They were the authors of the cooperative learning process, relating honey to organic compounds. They studied the carbon atom, and started functions. Molecular models aimed at the knowledge discussed (single/double/triple bonds, σ/π , hybridization/geometry, structures). For the second period, preparation of an extract by chromatography lead to the knowledge about properties of the compounds (which would occur in the fourth period), and applying the "Homologous Series" subproject. New groups were drawn (3rd period) in a motivating dialogical lesson. Trust was created between students and teachers within the learning process. In the last period, they investigated preliminary chemical results. The phenolic profiles were similar, with more differences in the Finnish honeydew and Portuguese floral. They studied isomers and completed functions. There were differentiated assessments for the construction of knowledge, including the use of technological tools. There was a surprising level of motivation amongst the participants. The implementation of PBL and student-centered approach is effective and powerful.

KEYWORDS: *Project-Based Learning (PBL). Student-Centered approach. Learn by doing. Collaborative work. Organic chemistry.*

Introduction

The educator is of fundamental importance in the mediation of knowledge, therefore, it can provide different pedagogical methodologies and innovative ways to mediate the teaching and learning process, arousing interest, creativity, critical thinking and student autonomy in the classroom.

One of the methodological proposals that seeks such contributions is known as Problem Based/Project Learning (PBL) developed in the late 1960s at McMaster University in Canada and later at the University of Maastrichna in the Netherlands:

The proposal is student-centered, where it seeks to learn for itself, giving the opportunity to direct its own learning and to investigate the scientific aspects involved in a given real or simulated situation that can present variable complexity (Sá & Brito, 2010). In addition, it presents as characteristics: thematic organization around problems; the interdisciplinary use of theoretical and practical components; the emphasis on cognitive development; the gathering of hypotheses, in the analysis of data and alternatives; and the active participation of the student in the classroom (DE OLIVEIRA, 2013; FARIA; SILVA, 2012; LARMER; MERGENDOLLER, 2012).

Within this proposal, the “Organic Chemistry” course was developed through the project "Characterization of honey by its profile in phenolic substances and evaluation of the antioxidant potential, focusing on the PBL method", within the scope of theoretical-practical approaches. This proposal has allowed the course list of contents to be taught in an applied and interdisciplinary way, as the project progresses.

Placing honey in the context of the project

Honey is a mixture of natural sweet-tasting substances produced by bees, from the collection of flower nectar (floral honey) or secretions from plant parts or from insect excretions that suck up parts of the plants (honeydew, extrafloral), and that bees transform and combine with the specific substances of their own organisms, being stored in the honeycomb until maturing. This is the general definition of honey from the Codex Alimentarius Commission (1990, as cited in Lianda, 2004) where all the commercially required characteristics of the product are described.

“Honey is a complex blend of carbohydrates, enzymes, aminoacids, acids, minerals, aromatic substances, vitamins, pigments, waxes and pollen grains” (Alvarez-Suarez, Tulipani, Romandini, Vidal, & Attino, 2009; Lianda, 2009). “Its composition, color, aroma and flavor depend mainly on flowering, geographical regions, climate and bee species”. “Some of the components are due to honey maturation, some are added by the bee and some are derived from the plants” (TOMÁS-BARBERÁN; MARTOS; FERRERES; RADOVIC; ANKLAM, 2001; LIANDA, 2009).

It is possible to infer that the honey composition is variable, and when there is a predominance of some floral species visited by the bees, the honey is classified as unifloral or monofloral and presents the designation of the species that generated it such as eucalyptus, orange honey, among others (LIANDA, 2004, 2009).

According to Tomás-Barberán et al (2001), the therapeutic activity of honey has been largely studied, mainly focusing on its antioxidant capacity, since it has polyphenols and flavonoids that confer antioxidant properties (LIANDA, SANT’ANA; ECHEVARRIA; CASTRO, 2012).

From these characteristics which honey presents and the possibility of adding several contents from the Organic Chemistry course with the preparation of the extracts, chromatographic analysis and the constituent substances, honey was the object chosen for the development of this project permeated by the PBL method.

Materials and methods

PBL Method

The project was applied in the “Organic Chemistry” course with 2nd year students of the Vocational Education integrated to Upper Secondary Education in Chemistry, in 2015, involving 30 students from the Federal Institute of Southeastern Minas Gerais - Barbacena Campus, MG, Brazil.

The PBL method consisted of many activities: cooperative learning; dialogic tasks; search for previous information on the topics before the classes; practical classes before the presented theory; use of technological tools: www.tagxedo.com; Facebook for communication between members and result presentation; assorted evaluations (in pairs, punctuating positive aspects and those that could be improved; in groups; participation), besides the written and individual test marked by the school; research in the topic literature that emerged during practical classes, with keyword learning generating a "cloud" through the use of the program www.tagxedo.com (individual); students as mentors, that is, students passing their learning on.

During the ongoing project (second period) it was necessary to elaborate a subproject or module ("homologous series of hydrocarbons and alcohols: applicability"), covering concepts of the properties of organic compounds, which would be taught in the fourth period following the conventional method.

Analytical Procedures

Honey Samples

The samples were stored in a refrigerator at 10°C until extraction time and are described in Table 1.

Table 1: Honey samples studied.

Sample	Flowering	Date	Region
MFRI	Finnish Honey <i>Rubus Idaeus</i> (raspberry)	2014	Finland
MFEA	Finnish Honey <i>Epilobium Angustifolium</i> (fireweed)	2014	Finland
MMF	Finnish honeydew	2014	Finland
MPR	Portuguese honey (rosemary)	2014	Portugal

Source: authors

Phenolic Substances Standard

Emphasis was given to those phenolics already described in the literature as possible chemical constituents of fruits and honey, besides their availability (commercial standards) in the laboratory. They are galic, synapic, syringic, ferulic, benzoic, 4-methoxycinnamic, 3,5-dihydroxybenzoic, 3-methoxycinnamic, meta-coumaric, ortho-coumaric, para-coumaric, cinnamic, 4-methoxybenzoic, protocatechuic and caffeic acids; and the flavonoids quercetin, kampferol, rutin, chrysin, myricetin, isoquercetin, naringenin, luteolin, apigenin and morin.

Sample extract preparation for chromatographic analysis

The phenolic substances were extracted from honey according to methodology previously described in the literature (LIANDA, 2004, 2009; LIANDA; CASTRO, 2008; TOMÁS-BARBERÁN et al, 2001).

The honey sample (about 50 g) was mixed with 250 ml of distilled water, adjusted to pH = 2 with concentrated hydrochloric acid, and stirred with a magnetic stirrer at room temperature until complete dissolution. The filtrate was agitated with about 75 g of Amberlite XAD-2 (styrene and divinylbenzene copolymer, pore 9 nm and particle 0.3-1.2 mm commercially obtained from Supelco-Bellefonte, PA, USA) and, then packed in a glass column (80 x 3.5 cm; open column chromatography - CC). The column was then washed first with acidified water (pH = 2, 100 ml), then with distilled water (about 150 ml) to remove all sugar and other polar constituents from the honey, while the phenolic substances remained in the column. The phenolic fraction adsorbed on the column was then eluted with methanol (about 350 ml). The obtained extract was concentrated until it was dry under reduced pressure in a rotary evaporator at 40°C (FISATON). After this step, the extraction procedure was carried out in which 15 ml of distilled H₂O were added to the extract, then the partition was made with two different solvents (each column in a solvent): a) ether (TOMÁS-BARBERÁN et al, 2001) and b) ethyl acetate (DA SILVA, 2004; LIANDA, 2004). After ten extractions (10 x 10 ml), the organic phases were combined, dried over anhydrous sodium sulfate and concentrated until it was dry on a rotary evaporator at 40°C. The extract was then dissolved in methanol and analyzed by TLC.

Chromatographic analysis by thin layer chromatographic (TLC)

Aluminum chromate sheets with 60 F254 silica gel were used. The eluent used as the mobile phase for evaluation of phenolic acids and free flavonoids comprised hexane: ethyl acetate: formic acid - 15: 24: 1, and for glycosylated flavonoids (chloroform: methanol: water: formic acid - 30: 18: 1 : 1) (LIANDA, 2009). The visualization of the substances was performed under UV light at 254 and 365 nm. There was a comparison of the R_f (retention factor) and the color and shape of UV spots.

Evaluation of antioxidant activity by TLC

Aliquots of the standards and each extract were applied to chromate sheets using as the mobile phase hexane: ethyl acetate: formic acid (17: 22: 1), and as a developer, 2,2-diphenyl-1-picrylhydrazila DPPH) at 0.2% in methanol, according to the method described by Ceruks, Romoff, Fávero, and Lago (2007). Substances with antiradical activity appear in the form of yellow spots on a violet background.

Results and discussion

The Analytical Program of the “Organic Chemistry” Course was adequate to the PBL method and diversification. The following are some episodes or activities that evidenced the application of the new method in this class.

Firstly, new ideas were presented, such as aspects of the PBL method and some dialogic tasks were performed. Group of students were formed randomly aiming at collaborative work among random pairs to get to know each other better, avoiding exclusion (for instance, prejudice against students who had previously failed) and also due to the fact that within the labor market it is not possible to choose colleagues. Students appeared to be very apprehensive and resilient because of the way the groups were formed.

Although the ideas were presented to the students, the title and object of study of the project (honey) were not initially disclosed, since this discovery would be part of the first activities. The first contact with the object of study occurred with the participation of two groups, separately, in the practical class regarding the first analysis to be carried out in the project. The researcher explained how to use the ultraviolet (UV) spectrophotometer, and the students still made sensorial analyzes, and the packaging labels were covered. Simultaneously, the rest of the class remained in class, and in groups, they observed a slide image about the object of study (honey), writing several words. At first, and individually,

they listed the common written words, and the different writings, so that later they could relate them to organic chemical compounds. Previously, by the conventional method, the class would follow, step by step, the content by theoretical means, using the whiteboard. In this class, the students began their learning inside the laboratory, visualizing and discussing the concepts in practice. Another novelty was the presence of collaborating professors (research professors and graduates).

One observation to be made is that by following this approach “surprises” arose during the lesson, that is, it was not possible to predict how it would occur rigorously, since students reflected on what they are doing, while discussing and asking. An example that can be cited during the first practical class is in relation to the use of methanol. Due to its toxicity, the students researched, individually, this organic solvent. They did, then, the first evaluation using a digital tool (www.tagxedo.com.br) in the cloud creation with keywords about the research carried out.

In the following class, it was proposed that the groups attending the 1st practical class taught the explanations, with their own words, to the other groups (group 1 taught to group 3, group 2 to group 4, group 3 to group 5, and groups 4 and 5 to the whole class), characterizing “Learning by doing” and peer orientations. Some students, also, voluntarily, taught a practical class about UV spectrophotometry to a group from the 7th period of Chemistry Degree (Teacher Training) in the “Instrumental Analysis” Course.

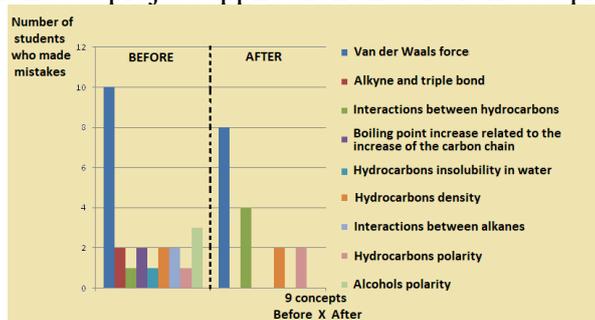
At this time, the UV spectra of the honey samples were obtained, an analysis that would not be part of the course syllabus. After this first contact with organic chemistry, there was a theoretical lecture about introduction to the carbon atom. At this point of the project development, students already have the knowledge that honey refers to, the object of the study. Examples of organic compounds that may form part of the composition of honey (according to indications resulting from UV analysis, and previous knowledge of the teacher) were used in this lesson to present simple, double and triple covalent bonds, therefore sigma and pi. Some guidelines were given so that the students could prepare themselves by researching for the next lesson: the concepts regarding the carbon atom, in addition to those previously mentioned. Then, the next theoretical class was directed to the application of the concepts studied by the students (at home) about the carbon atom. With the use of molecular models (traces and orbital), the study of hybridization and geometry of the Carbon sp³ was carried out. The sp² Carbon study was started. The novelty in this lesson is not the use of molecular models, since it was already used before, but rather the

handling by the students (not only by the teacher), before and during explanations about it. The time involved to finalize one aspect of the course syllabus seems to be greater, but the result is better.

After the practical classes, the preparation of extract for chromatographic analysis (involving column chromatography - CC, contents for the “Instrumental Analysis” Course, in the 3rd year) was started (second period). Discussions arose about physical-chemical properties i.e. contents for the “Organic Chemistry” Course, but it would be in the fourth period. The idea of creating a module, as a subproject, has been introduced, including concepts related to these properties, such as, polarity, solubility, boiling point, melting point, intermolecular forces. The module titled "Homologous series of hydrocarbons and alcohols, applicability" was prepared by the researchers-professors and Chemistry undergraduates. Several practical classes (or theoretical-practical) were carried out, with a previous theoretical study of the students, involving specific subjects: "Study of some properties of alkanes, alkenes, alkynes and alcohols". During these lessons, students' participation was constant and motivation from both students and team members was evident (in some moments students led the discussions and taught classes). At the end of the practical classes, a questionnaire was applied to be answered in groups on all the properties visualized and discussed in the laboratory. There were, after the practical process, theoretical lectures on all properties, besides nomenclature of these organic functions, to organize the content taught.

The questionnaire was redone by the students, in pairs, after discussions in the theoretical classes. There was a clear evolution of 9 concepts: 5 improved 100%, 1 in 20%, the most difficult, 1 remained, 2 worsened in percentage, but represent the smallest share of difficulties, see Figure 1 below.

Figure 1: Graphs comparing the number of students who made errors in each concept at the beginning of the subproject application and after the subproject application.



Source: The authors' data.

The subproject takes place during the full 2nd period, so its content was part of the bimonthly evaluation required by the Institution. The test scores were 1/3 of the students ≥ 80 , 1/3 ≥ 70 , 1/5 ≤ 60 . The average 2/3 ≥ 80 , and no one had to retake exams.

New groups were organized randomly (3rd period) in a motivating dialogic lesson, since there was an improved acceptance of the approach, and the students were already more confident in themselves, in the learning process and in the team of teachers. After that, some dialogic activities were carried out with the purpose of making each student aware of their importance to the group and the importance of their colleagues to accomplish activities successfully. Of course, the dialogic activities also helped students to become more prepared for the job market, where contact with the other people is fundamental and indispensable.

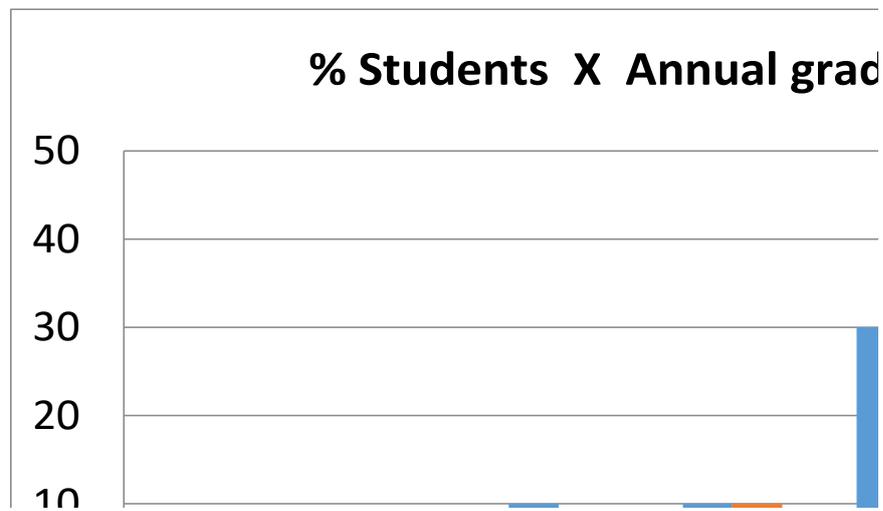
The students themselves were the authors of the teaching and learning process cooperatively, relating honey to organic compounds. The studies were deepened with the use of molecular models (Csp² / sp / geometries).

In the practical classes, after following the preparation of the extracts, discussing the phenolic compounds and possible isomers present in the samples (such as morin / quercetin), this topic and the organic functions were finalized.

During the whole process, emphasis was placed on prior studies, classroom activities, and differentiated assessments for knowledge construction. The previous study provided a higher student performance during the classes. The classes became more productive, dynamic and contextualized, since the doubts about the subject studied provided constructive debates.

In the last period, the groups were defined by the students themselves, who appeared to be more mature. They even had difficulty to divide themselves in new groups, because they felt close to one another and wanted to be a single group. Chemical results were sought, whose study should continue later.

The annual grades demonstrated that the PBL approach can be a valuable tool as contribution and innovation to the teaching and learning process (Figure 2).

Figure 2: Chart of percentage of students' X annual grades (2015).

Source: The authors' data.

This project contributed to the students acquiring ability to act in the job market in a critical, practical and reliable way, as well as learning the teamwork spirit.

The results surpassed expectations since the most dynamic, dialogic and creative classes drew the students' attention who reciprocated with interest, participation and teaching and learning performance process. Despite the students' initial resistance, they gradually gained confidence in the teaching method, in the teachers, and in themselves. They visibly became more communicative, motivated, committed and critical than at the beginning of the implementation of the PBL method.

The students gave testimonies in each period, positively, but admitted in the end, that they felt more confident at the 3rd period. Here are some testimonials: "I am really enjoying the lessons because with this approach learning has become easier. Learning by doing has made the content learned and not memorized". "We learn to work in a team, to respect and to help". "I concluded that with this method it is possible that before we get to the classroom we already know the subject, then it becomes easier to understand when explained". "This practical-class method before theory is very interesting and cool, and having to research to learn is also good". "Well, I am not going to lie, at first I did not agree with that, I thought it would harm us and we would be out of line with the content. But I realized that it was not bad, and that instead of leaving outdated and impaired, we will have more knowledge and better training".

Chemical analysis

The acquired material allows the continuation of the study as a perspective. The classical processes based on TLC and open column (CC) were used for the separation and purification of the natural products of interest (phenolic substances).

Ultraviolet spectrums of honey samples

Initially, a study of the UV absorption spectrum of the honeys (solubilized in methanol) allowed to make some considerations regarding the structures of the substances to be identified. The analysis of these spectra allowed to verify localized absorption between 250-360 nm, an absorption range that suggested the presence of phenolic derivatives, including phenolic acids (benzoic acid and its derivatives, cinnamic acid and its derivatives) and flavonoids (flavones or flavanones). All honeys showed similarities in their UV spectrum.

Analysis of honeys extracts

Many phenolic substances present in the samples did not coincide with the standards used, and there is no possibility of indicating identification by the method adopted. Preliminary results are shown in Table 2.

Table 2: Phenolic substances identified by TLC.

Sample	Phenolic Substances
MFRI	Acids: <i>para</i> -coumaric, benzoic, ferulic or synapic, 3-methoxycinnamic or 4-methoxybenzoic, Flavonoids: maybe kampferol, maybe isoquercetin, naringenin
MFEA	Acids: 3,5-dihydroxy-benzoic, cinnamic, ferulic or synapic, 3-methoxycinnamic or 4-methoxybenzoic acid, <i>meta</i> or <i>ortho</i> -coumaric Flavonoid: maybe isoquercetin
MMF	Acids: <i>para</i> -coumaric, benzoic, cinnamic, ferulic or synapic Flavonoids: maybe kampferol, apigenin
MPR	Acids: syringic, ferulic or synapic, 3-methoxycinnamic or 4-methoxybenzoic, <i>meta</i> or <i>ortho</i> -coumaric

Soucer: authors

Antioxidant activity of honeys extracts

Most antioxidant methods used involves the generation of oxidant species, usually free radicals, and their concentration is monitored in the presence of antioxidants that abduct them. The DPPH molecule is considered a stable free radical by having a free electron that can delocalize throughout its structure. This resonance effect that the DPPH molecule presents results in a dark violet coloration in ethanol or methanol solution. When the DPPH solution is mixed with some substance that can donate a hydrogen radical, then the reaction generates its reduced form with loss of violet coloration (although a yellowish colored residue is expected to appear due to the presence of the picryl group).

Finnish weed honey was the one that showed the greatest yellowish coloration in its spots, and even more when extracted with ether. This data can enrich the discussion regarding the structures of the possible phenolic constituents of this honey, taking into account the presence of substituent hydroxyl groups in the aromatic ring, and their position, such as the ortho position favors the stabilization of the free radical and the meta position, and also the methoxy group will not present the activity. In this context, there is a tendency to consider a greater probability of the presence of ortho-coumaric acid and, perhaps, not the target, and is also justified by the presence of 3,5-dihydroxybenzoic acid. Portuguese honey, however, did not reveal yellow staining in its spots, which put into question exactly the inverse possibility of its phenolic substances being preferentially methoxylated.

Final considerations

In order to promote students' meaningful learning, the use of the PBL method in the teaching of Organic Chemistry has proved to be efficient with respect to the formation of critical, curious, and participatory subjects engaging pleasantly in Chemistry classes. This implementation surpassed expectations, with dynamic classes, drawing students's attention who in return showed interest and engagement. There is evidence of motivation on the part of students and staff. They can be emphasized: the construction of collaborative knowledge, with respect to individuality; confidence generation among students, in the learning process and in the teacher; students reflecting on what they are doing; skills development of accomplished teamwork and management. The study of organic compounds in a way correlated with a daily food, and still being isolated from the matrix

(honey) by analytical techniques led to significant learning, as well as the contribution to the scientific community (educational, chemical, beekeeping, biological and nutritional).

The European honeys showed similar phenolic profiles among the samples, being preliminary results. The phenolic acids indicated were: ferulic or synapic (in all samples), 3-methoxycinnamic or 4-methoxybenzoic (in floral samples), meta or ortho-coumaric, para-coumaric, benzoic, cinnamic, 3,5-dihydroxybenzoic (in the weed sample) and syringic (only in the Portuguese rosemary sample). The possibility of the presence of flavonoids appears only for Finnish samples, isoquercetin (in floral samples), kampferol, naringenin (in the raspberry sample) and apigenin (in the honeydew sample). Preliminary analysis of antioxidant activities by TLC using as DPPH developer revealed that the honey that showed the most activity was the Finnish weed. In general, the Finnish honey had more activity than the Portuguese one, perhaps suggesting that their phenolic substances are hydroxylated to the detriment of methoxylates, whereas Portuguese honey is the exact opposite. It seems an interesting result, to differentiate it from the Finnish honey, since the flora and the climate of these countries are different.

Finally, the implementation of student-centered and project-based learning (PBL) approach in the study is effective and powerful.

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