

Investigative and collaborative experimental activities using simple purification of curcuminoids by column chromatography

Actividades experimentales investigativas y colaborativas mediante purificación simple de curcuminoides mediante cromatografía en columna

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Resumen

Este trabajo describe el uso de la cúrcuma en la enseñanza de conceptos y técnicas de laboratorio de química orgánica a estudiantes de clases introductorias de cursos de licenciatura en química, grado en química y química industrial, así como a estudiantes de farmacia e ingeniería química de la Universidade Federal Fluminense, en Niterói/RJ/Brasil. Considerando las actividades de investigación y colaboración con Vygotsky, los conceptos discutidos fueron productos naturales cotidianos, química verde, purificación y separación de sustancias orgánicas, interacciones moleculares, técnicas de extracción de compuestos orgánicos, cromatografía en capa fina y en columna. El trabajo se aplicó durante cuatro clases de 3 horas de duración a 31 estudiantes de 7 clases de Introducción al Laboratorio de Química Orgánica de la carrera de Química y 43 estudiantes de 4 clases de Química Orgánica Experimental de la carrera de Farmacia e Ingeniería Química, en los años 2022 y 2023. La metodología del proceso de enseñanza-aprendizaje involucró 4 momentos/clases: a) discusiones sobre química orgánica; b) extracción discontinua de cúrcuma y cromatografía en capa fina; c) cromatografía en columna; y d) discusiones sobre resultados y proceso de aprendizaje. Luego de la secuencia de clases, se pudo observar la motivación de los estudiantes con la facilidad para separar los curcuminoides y la comprensión de la relación entre conceptos teóricos y práctica, así como la comprensión de la importancia de la baja producción de residuos.

Palabras clave: química orgánica; enseñanza experimental; aprendizaje; productos naturales; clases introductorias de laboratorio; interdisciplinaridad.

Abstract

This work describes the use of turmeric in the teaching concepts and laboratory techniques of organic chemistry to students in introductory classes of licentiate, bachelor's and industrial chemistry courses, as well as to pharmacy and chemical engineering students at Universidade Federal Fluminense, in Niterói/RJ/Brazil. Considering investigative and collaborative activities and Vygotsky theory, the discussed concepts were everyday natural products, green chemistry, purification and separation of organic substances, molecular interactions, organic compound extraction techniques, and thin-layer and column chromatography. The activities were applied for four 3-hour classes for 31 students in 7 introductory organic chemistry laboratory classes for Chemistry and for 43 students in 4 experimental organic chemistry classes for Pharmacy and Chemical Engineering courses, in the years 2022–23. The teaching-learning process methodology involved 4 moments/classes: a) discussions about organic chemistry; b) discontinuous extraction of turmeric and thin-layer chromatography; c) column chromatography; and d) discussions about results and the learning process. After the sequence of classes, it was possible to observe the students' motivation with the ease of separating curcuminoids and the understanding of the relationship between theoretical concepts and practice, as well as the understanding of the importance of low waste production.

Keywords : organic chemistry; experimental teaching; learning; natural products; introductory laboratory classes; interdisciplinarity.

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Introduction

The teaching of chemistry at both secondary and higher education levels aims to involve students in the learning process, in addition to promoting the understanding of fundamental concepts through practical and applied activities. Chemistry is a very abstract field of science, having, for example, molecular nomenclatures and formulae that are often complex, causing students to distance themselves from focusing on their learning objectives, making the teaching-learning process difficult; in addition, the teacher must seek out and learn new methodologies and strategies to further promote learning (Treagust et al., 2000; Albano & Delou, 2023; Adu-Gyamfi & Asaki, 2023). Often, students have a preconceived view of a certain phenomenon due to their understanding of the topic, built over the years (Winarni, et al., 2022). A great desire of educators is that their students not only learn the concepts discussed in their classes, but that they also apply them in practice, whether in the professional field or in their daily lives. Thus, a facilitator of the teaching-learning process would be to balance theoretical teaching with practice, while considering the role of the teacher and student in this educational process (Wrenn & Wrenn, 2009). Thus, as pointed out by several studies described in the literature and recommended by educational bodies in different countries, exploring laboratory techniques is essential to developing analytical skills, encouraging deductive reasoning, and illustrating the relationship between chemical theory and practical application (Ntawuhiganayo & Nsanganwimana, 2022; Shana & Abulibdeh, 2020; Santos & Menezes, 2020; Novais, 2018). Therefore, an important issue that arises in teaching chemistry or science, is the need to make the source material relatable to students in their daily lives, which involves scientific and social knowledge aimed at training that leads to the development of skills and competencies in students so that they are capable of a critical view of events in their personal, professional, and social lives. We believe that there are many pedagogical paths that teachers can take to promote motivation, understanding of theoretical and practical concepts, skills, and competencies for critical and reflective training, as the literature has demonstrated through various studies and debates (Seabra et al., 2023; Bilar & Coutinho, 2021; Barbosa et al., 2021).

One of these methodologies is Investigative Learning, which requires the teacher to create a conducive environment for students to build their own knowledge. Chemistry is a field of science that uses many experiments to discuss its concepts and symbols, which can stimulate student interest in learning about this knowledge and is also a very interesting time to develop investigative activities. Many studies have demonstrated how learning through this methodology can motivate students to participate more actively in classes, thus facilitating their development, skills, and competencies (Ribeiro et al., 2022; Lima et al., 2022; Menezes & Farias, 2023; Leite, 2018). Considering that collaborative learning can be defined as a practice in which individuals or groups help each other, without hierarchy, to achieve common objectives in different contexts, including educational ones (Nascimento et al., 2023; Pozzi, Manganello & Persico, 2023; Muñoz-Osuna et al., 2014), we believe that in investigative learning there can be collaboration between individual and collective knowledge and the history and culture of each student and teacher for everyone's intellectual development. Marques & Rosa (2023) discuss, among other issues, that a person's development can be linked to the relationship between development and instruction and to the collaborative action of another person. They also emphasise that Vygotsky shows

that instruction can lead to development if carried out collaboratively, whether by adults or between peers. As Novita et al. (2020) recalls, considering Vygotsky's theory, when there is socialization and interaction between students and teachers, involvement in activities is facilitated, allowing them to be part of a shared cultural community, which would favor cognitive development in a collaborative way.

Therefore, it would be possible to bring theoretical concepts closer to practice through the investigative and collaborative process to favor the teaching-learning process of organic chemistry using students' everyday products, such as food.

The Department of Organic Chemistry at Universidade Federal Fluminense (UFF) offers experimental classes for students studying Chemistry and Pharmacy. The discipline "Introduction to the Organic Chemistry Laboratory", for example, is offered in the first semester of the Chemistry program, and for many students it is their first contact with experimental classes. These classes aim to address general concepts in organic chemistry such as solubility, intermolecular forces, purification and separation of substances through extractions, and planar chromatography such as paper, column and thin-layer chromatography (TLC). The discipline "Experimental Organic Chemistry V" aims to address a greater number of purification and separation techniques for organic substances, covering the same concepts as the previously mentioned discipline, but using continuous and discontinuous extraction experiments, such as simple, fractional and steam distillations and chromatography techniques (TLC and column), but offered in the third period of the Pharmacy program. In addition to these chemical contents, these concepts can be contextualised, for example, through green chemistry, the environment, natural products, food, and industry. As in all practical disciplines offered by the Department of Organic Chemistry, the experiments seek to integrate theoretical concepts of organic chemistry with practical applications, encouraging deduction and analytical thinking. In this sense, the chemistry of natural products is fascinating for contextualisation and motivation in the chemistry teaching-learning process.

The roots of the species *Curcuma longa* L. (*Zingiberaceae*) are widely used in the food industry, either as a yellow dye or in the composition of some seasonings such as curry. This species has a series of pharmacological activity studies both *in vitro* and *in vivo*. Turmeric is used in traditional Chinese medicine in the treatment and prevention of various diseases such as cancer, cough, diabetes, arthritis, diarrhea, inflammation, psoriasis, hepatobiliary diseases, skin diseases, gastric ulcers, and peptic ulcers (Iweala et al., 2023). In Brazil, there is already a herbal medicine available for the treatment of osteoarthritis and rheumatoid arthritis. Turmeric rhizome has three main pigments responsible for its yellowish color: curcumin **1**, desmethoxycurcumin **2** and bisdesmethoxycurcumin **3**. Figure 1 shows curcuminoids **1-3** and their analysis by TLC.

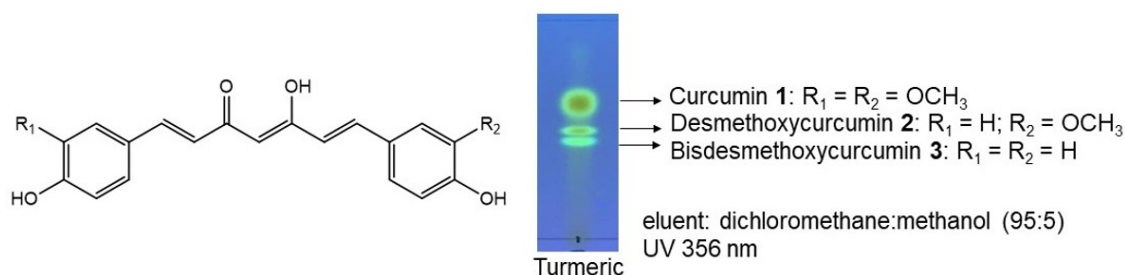


FIGURE 1 Showing the curcuminoids **1-3** and their analysis by TLC.

The use of curcuminoids for teaching chemistry through experiments has been widely studied, motivated by their relevance in terms of application and scientific interest. Their analysis and separation using chromatographic techniques not only provide an opportunity for the practical application of theoretical knowledge covered in the classroom, but also introduce students to the vast field of natural product analysis. The use of turmeric pigments from food to discuss laboratory techniques such as extraction and thin-layer chromatography through a sequence of experiments, for example, enabled students in the second and third years of education to understand the relationship between theoretical and practical concepts (Fagundes et al., 2016 & 2018). Other authors have already used turmeric or its derivatives to design laboratory activities to isolate secondary metabolites and for microwave-promoted synthesis experiments to teach organic chemistry (Hakim et al., 2016; Mullins & Prusinowski, 2019). Another important approach in teaching chemistry is the discussion about reducing the scale of experiments to meet the principles of green chemistry, and by reducing the amount of solvents, reagents, and other chemical products leading to a reduction in waste generation. As an example, we can mention the work of Ciaccio & Hassan (2020) in which they used column chromatography to separate carotenoids and chlorophyll from the extract of green leaves like spinach, among others, using a Pasteur pipette (0.75 g of silica). In other work, more recently, Ciaccio & Ak (2022) used small-scale dry column vacuum chromatography to successfully isolate essential oil.

Due to our interest in improving the teaching of chemistry, this work aims to present an investigative and collaborative didactic approach to discuss theoretical concepts and laboratory techniques in chemistry by separating curcuminoids from the rhizome of *C. longa*. In this way, we seek to facilitate the understanding of experimental techniques and theoretical concepts of chemistry and promote in students a critical view of the importance of science in their personal, professional, and social lives. In this sense, in addition to discussing chemical concepts such as molecular interaction, solubility and purification techniques for organic substances through everyday products, we seek to introduce the theme of green chemistry by reducing the quantities of reagents in experiments, thus showing the importance of reducing waste for better pollution control, in addition to discussing the significance of natural products through everyday foods. Furthermore, we will highlight the educational value of the experience in promoting students' practical skills and abilities by the use of experiments considering the investigation and collaboration methodologies.

Methodology

This work can be considered qualitative and action research, since according to Tako & Kameo (2023), it does not seek to analyse the results in a statistical way but seeks to develop from the interaction between researchers and members of the investigated situations. The research environment took place in 2022-2023 in 7 classes of the discipline 'Introduction to the Organic Chemistry Laboratory' and 'Experimental Organic Chemistry V' in the Chemistry, Pharmacy and Chemical Engineering programs. The total number of students was 74 and groups of up to 2 students were formed to carry out the work.

TABLE 1: Disciplines, years, semesters of the classes and number of students.

Discipline: Introduction to the Organic Chemistry Laboratory. Chemistry Program		Discipline: Experimental Organic Chemistry V. Pharmacy and Chemical Engineering Program	
Year/semester/ number of classes	Number of students	Year/semester/ number of classes	Number of students
2022/2/2	16	2023/1/4	43
2023/1/2	15		

Didactic approach

The didactic method involved investigation through both bibliographical research and experimentation and took place during 4 sessions of 3-hour classes each. In this sense, four classes were used, during which students discussed, based on literature and other sources, such as newspapers and magazines, topics involving the chemistry of natural products, separation, and purification methods, following the principles of green chemistry and theoretical and experimental organic chemistry. The students were instructed to investigate through technological means of communication how it would be possible to analyse and purify the curcuminoids present in saffron. Furthermore, the students' investigative work (Ribeiro et al., 2022; Lima et al., 2022; Menezes & Farias, 2023; Leite, 2018) occurred collaboratively (Nascimento et al., 2023; Pozzi, Manganello & Persico, 2023; Muñoz-Osuna et al., 2014), since groups of 2 students had to propose a solution to a problem and execute it based on discussions between them and the other groups on the topic throughout the process.

Sequence of Classes (four classes of 3-hours each)

First class: This class discussed green chemistry, extraction and purification of organic compounds, and chemical properties of molecules, such as intermolecular forces. The chemistry of natural products was also discussed, using as an example several plants from our daily lives, such as turmeric. In this case, in addition to discussing their use as food and medicine, their properties, forms of extraction and purification of their main components, curcuminoids **1-3**, were discussed. Students were separated into groups of two students and encouraged to develop a proposal by literature investigation for the separation of curcuminoids **1-3** from turmeric rhizome powder. The teaching resources were the use of PowerPoint on laboratory techniques, electronic equipment for online bibliographic research, and discussions.

Second class: In this class, students should, based on their proposals, choose the best eluent for separating the curcuminoids present in turmeric rhizome powder by thin-layer chromatography of a dichloromethane:methanol solution (99:1) or ethanol as solvent for a period of about 20 minutes. The presence of curcuminoids must be analysed by solving the crude extract on TLC plates (3 × 6 cm) to determine the best solvent to be used in separating the three main components **1-3** through a chromatographic column. The chromatographic plates must be observed with the naked eye (white light), under a UV lamp (at 365 and 254 nm), under a black light lamp (400 – 320 nm), and 20% H₂SO₄ solution in ethanol (Fagundes et al., 2016). The teaching method at this moment is investigative and collaborative experimental research, based on discussions between groups about the proposals prepared by them.

Third class: Students should, based on their proposals and the choice of eluent, separate the curcuminoids present in the turmeric rhizome powder by column chromatography. However, we first describe the best procedure for this experiment: the ethanolic extracts obtained previously must be purified in a 25 cm long and 1 cm diameter chromatographic column using 2.5 g of silica. The chromatographic column should be packed using a dichloromethane:methanol (99:1) solution and curcuminoids **1-3** are separated into 15 fractions of about 3 mL each. This eluent is used until the first curcuminoid comes out. From then on, a dichloromethane:methanol solution (98:2) should be used as eluent. The three main components are separated into up to 15 fractions of 3 mL each. The fractions must be analysed by thin-layer chromatography, as carried out in the second class. Again, the teaching method at this moment is investigative and collaborative experimental research, based on discussions between groups about the proposals prepared by them.

Fourth class: Each group of students presented their results, discussing the difficulties encountered and contextualizing theory and practice and chemical and environmental concepts. The teaching resources were the use of PowerPoint on the experimental results obtained, a brief report and discussions. Once again, the discussion between the groups about what was investigated theoretically and experimentally allowed conclusions about the solution to the problem to be reached in a collaborative way.

Material and hazards

Ethanol, methanol, ethyl acetate, and dichloromethane were purchased from Vetec. TLC silica plates Micro/AL F-254 SiliCycle were used for TLC analyses. Turmeric was purchased at the market. Sulfuric acid 20% solution in ethanol, iodine, and a 254 and 365 nm UV lamp were used to detect the curcuminoids **1-3**. The rotatory evaporator used for distillation was Rotavapor R-114 from Buchi. The dichloromethane, hexane, ethyl acetate, methanol, and ethanol used in the experiments are volatile and flammable organic solvents, therefore all work with this solvent should be conducted in the fume cupboard. These solvents are harmful if ingested, inhaled, or come into contact with skin. UV lamps must be handled with UV eye protection goggles because the UV radiation can damage eyes and cause skin cancer. Throughout all the procedure's lab coats, gloves, and safety glasses should be used.

Results and Discussion

Initially, after bibliographical research and discussions on the chemistry of natural products, green chemistry and the techniques necessary to extract, analyse and purify by thin-layer and column chromatography, respectively, the group of students presented their proposals for the extraction and obtaining of curcuminoids from turmeric. Several proposals were made, with discontinuous liquid-liquid extraction with ethanol being chosen to obtain the turmeric extract, thin-layer chromatography for analysing the extract and column fractions, and chromatographic columns as a purification method. For thin-layer chromatography and column chromatography, different eluents were selected, with a solution of methanol or ethanol in dichloromethane being preferred. It was possible to observe at this point the students' motivation in proposing a solution to the problem and the importance they gave to researching the literature for the most appropriate choices of eluents for pigment analysis and purification, and for selecting the most suitable extraction method: fast execution time, quantities to be used of turmeric sample, solvents, silica, and chromatographic plates and

taking into consideration the need for low waste production. Furthermore, it was possible to perceive the consideration they had regarding the importance of using small quantities, and how research into the literature and collaboration between the groups to solve the problem, based on their proposals, led to common sense among the groups.

In the second class, based on the proposals and discussions, students easily detected curcuminoids **1-3** by experimental investigation using TLC. At that moment, the students realized the difference and the greater or lesser efficiency of the developers for separating the substances, as well as the best eluent to be used for the separation in the chromatographic column. From observing the students' dialogues in the investigative process, it was possible to see that they considered experimental investigation important for understanding the correlation of theoretical concepts with experimental results. Likewise, discussions throughout the experiments collaboratively allowed a better understanding of what was occurring for the purification of curcuminoids **1-3** and the chemistry involved. Figure 2a shows the results of the TLC analyses from some students.

In the third class, most students, based on the results obtained previously and the debates, chose to carry out the separation of curcuminoids **1-3** using a dichloromethane:methanol (99:1) solution as eluent. At this point, some groups had better results in separating pigments than others. Figure 2b – e shows some results obtained by the students in separating the pigments.

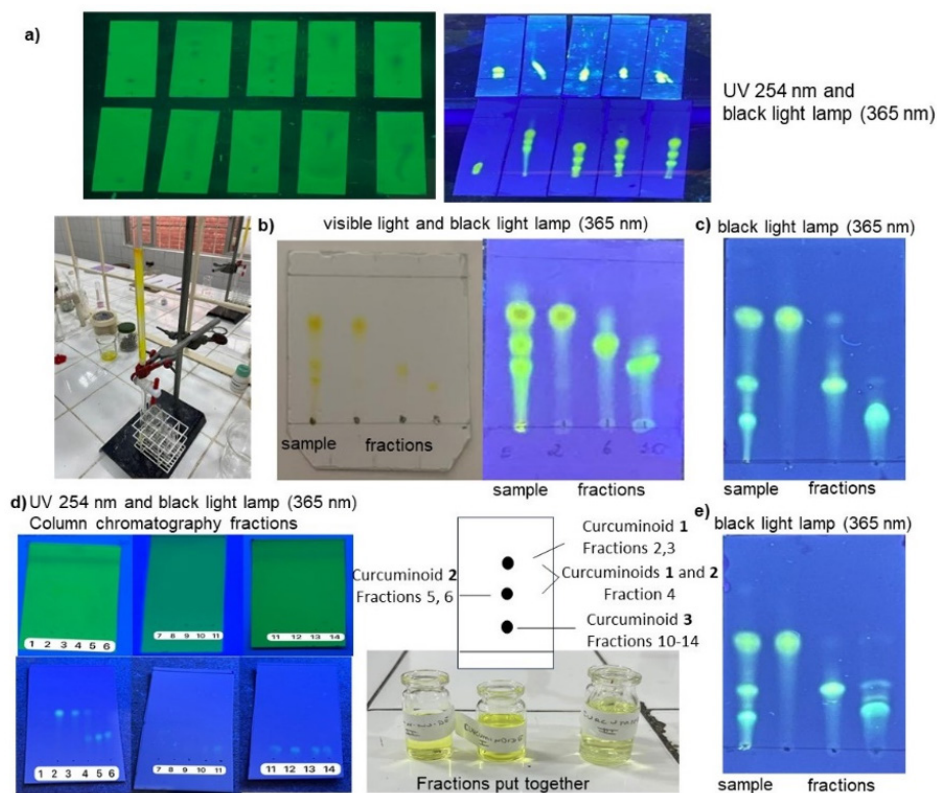


FIGURE 2 a) TLC analyses results described by groups using various eluents; b) column chromatography; c) to e) analysis of column results described by groups.

Thus, it was possible to perceive the importance of the relationship between the quantities of samples and their retention factors, the size of the chromatographic column, and the volume of the fractions, since smaller volumes of the fractions provided

better results. The students also realised the importance of eluents in the purification process. Considering the results obtained by the students, we can also consider that the analysis of curcuminoids by thin-layer chromatography, as well as their purification in a chromatographic column under the experimental conditions carried out (eluent dichloromethane:methanol and small amounts of samples - 25 mg of turmeric), are simple and easy to perform, unlike work already reported in the literature, as small amounts of sample are not used, or the three main components, curcuminoids **1-3**, cannot be easily isolated. Once again, discussions between the groups during the process about their experimental investigation results, collaboratively allowed them to preliminarily reach conclusions about difficulties encountered and better solutions to the problem.

Finally, in the last class, the students presented their results orally and debated numerous aspects of knowledge, such as waste production, costs of materials used, the environment, and organic chemistry. Once again, it was possible to realise the importance of investigative research and collaboration in discussions to perceive how important it is to understand theoretical concepts and bibliographical research to reach the desired objective, which in this case was the separation and purification of curcuminoids **1-3**. Figure 3 shows some moments of the students in the classes.



FIGURE 3 Students during the moments of discussions and experimental investigations.

As can be seen, the methodology always involved the investigative process (Ribeiro et al., 2022; Lima et al., 2022; Menezes & Farias, 2023; Leite, 2018), as to propose a solution to the problem, information for experimental investigation was sought in the literature. On the other hand, the investigative process took place collaboratively (Nascimento et al., 2023; Pozzi, Manganello & Persico, 2023; Muñoz-Osuna et al., 2014), since when researching the literature and experimentally executing the proposals, there is an exchange of knowledge between students, which favors socialization and the teaching-learning process, as suggested by Vygotsky (Marques & Rosa, 2023; Novita et al., 2020).

The perception of learning about theoretical and experimental concepts was observed during the discussions, as well as through a practical experimental exam, in which the groups had to confirm the presence of a certain substance in a sample. In general, the students chose the appropriate eluent and confirmed the presence of the substance by thin-layer chromatography. Figure 4 shows one of the results obtained by a group, which shows the presence of eugenol in an unknown sample; they even noticed from TLC that the standard was slightly contaminated. In this way, it is possible to affirm that the students were able to transfer the knowledge acquired to a real problem through investigation.

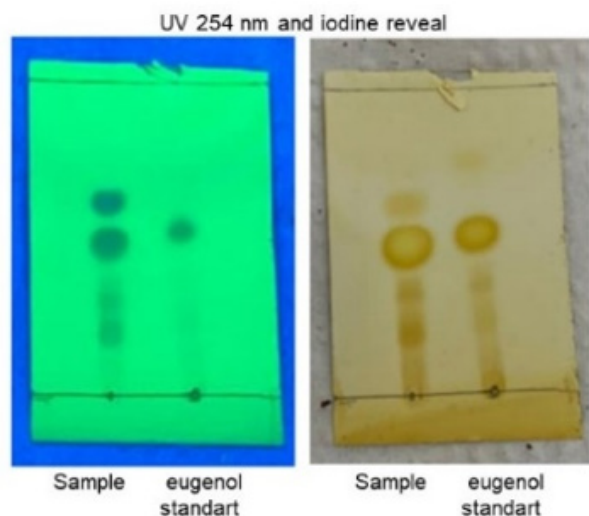


FIGURE 4 Some results of students from practical exams using TLC.

In addition to the perception of learning through the experimental investigation described previously, at the end of all period students were encouraged to respond voluntarily and anonymously to a questionnaire available on Google Form. Around 20 (27%) students responded to the questionnaire. The form contained 13 questions: the first three of which sought to identify the course and class to which the student belonged and if they took the technical course; the next two sought to find out if they had already had contact with chromatography experiments in high school; the subsequent six sought to find out the students' understanding of the experiments, whether they knew how they chose the eluents, whether they were able to separate the curcuminoids and identify what they were, and how they analysed the fractions in the columns; and last two questions to know if they liked the teaching methodology and if they had comments and suggestions.

From the answers we can say: a) the students who responded were divided equally between the classes and did not study in a technical course; b) over 80% of them had not had contact with chromatography experiments until then; c) the vast majority, above 90%, explained how they decided the solvents, how they separated the curcuminoids, and what they were; and d) 100% of students liked the experiments and many praised the methodology used. In this way, we can say that the application of the investigative and collaborative methodology favoured the understanding of the contents and the possibility of resolving new problem situations. Figure 5 shows some student comments taken from the questionnaires.

- The chromatographic column aims to separate and isolate substances present in a sample
- System DCM:MeOH (99:1) and then DCM:MeOH (98:2)
- An experimental class was previously carried out testing 5 eluents and mixtures of these in different concentrations in search of the best elution and separation of substances using the thin layer chromatography technique. Where better elution occurred using concentrations 99:1 and 98:2 of the dichloromethane:methanol mixture.
- To check whether the separation was effective, a test was carried out using thin layer chromatography. The sample after extraction and the substances present in the second, sixth and tenth test tubes collected from the column were deposited there, and then these were eluted with FM dichloromethane:methanol 98:2 and compared.
- The practices were really cool, the way forward is to have more activities that actually represent everyday life, that is, a discipline that is focused on experiments that go hand in hand with life and not just "test tubes".
- I loved the way the discipline was separated. We managed to carry out all the experiments according to the schedule and the classes were very productive. I found it very worthwhile that the theoretical class was carried out with practically all the content of the semester, as this way, the practices were better utilized. Congratulations to the teacher and thank you very much for your dedication!

FIGURE 5 Some comments from students about the classes.

Conclusions

In this work it was possible to apply an investigative and collaborative experiments using technological resources, such as PowerPoint, electronic devices, and the internet for the theoretical investigation and discussions for critical and reflective training, as well as experiments on extraction and chromatography for the analysis and purification of natural products to compare theory and practice through experimental investigation and collaboratively. To this end, groups of students proposed and carried out experiments in

order to investigate the purification of organic substances present in plants, in addition to collaboratively discussing the topics covered in the search for a solution to the problem that was the isolation of the three curcuminoids from the turmeric root, using small amounts of chemicals.

It was possible to discuss topics such as the importance of the environment and the reduction of waste production, the importance of natural products as a source of food and substances with important biological potential, and how the theoretical concepts of a given science are directly correlated with experimental practice and with our daily lives. Specifically, regarding chemical and experimental concepts, the experimental sequence involved the use of turmeric as a standard method for obtaining extracts by non-continuous extraction; the use of a chromatographic column for extracts; and TLC for analyses of extracts and purification fractions. Furthermore, students were able to develop an understanding of theoretical aspects such as intra- and intermolecular hydrogen bonds, tautomerism, resonance, solubility, and polarity.

Finally, it was possible to perceive the potential of using investigative and collaborative research as a method for improving the teaching-learning process.

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