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# SCIENCE PRACTICUM ACTIVITIES THROUGH SEPARATION OF LEAVES PIGMENTS BY PAPER CHROMATOGRAPHY IN JUNIOR HIGH SCHOOL

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**Abstract:** The material on mixture separation is still challenging for students to grasp; students still do not fully understand the concept of chromatography. One way for students to gather information is to plan and carry out experiments or practicum activities. This study aims to provide students with experience in separation methods aligned with the learning outcomes. The method used is the development of an experiment-based practicum on the separation of mixture materials, which was carried out with 7th-grade students at the junior high school level. The result of paper chromatography can separate pigments present in leaves. The pigments found in *Iresine diffusa* leaves are chlorophyll a, characterized by the green color, chlorophyll b, characterized by the yellow-green color, and carotene, characterized by the orange color. However, the polarity of the solvent does not influence the Rf value in leaf pigments, as the range of Rf values remains consistent with other research results, even though the components of the solvent used are different. Students' responses regarding comprehension of the material and motivation to learn during this practical activity also show high levels, with an average of 74.43%.

Keywords: Experiment-Based learning, Paper chromatography, Students' comprehension, Students' motivation

## INTRODUCTION

Science is a subject that can be taught at various educational levels, from the fundamental to the advanced. Natural Sciences involve the examination of the natural world. Systematically, science goes beyond simply acquiring knowledge in the form of facts, concepts, or principles; it also encompasses the process of discovery (Pratiwi, 2022; Liana et al., 2023). When studying science, much material can be taught through the experimental or practicum method. Practicum activities offer a means to overcome challenges in science learning, including difficulties in comprehending material, establishing connections between concepts, understanding formulas, and applying them in problem-solving (Rini & Aldila, 2023). A characteristic of the practical method is the use of a scientific approach.

The steps within the scientific approach encompass observation, inquiry, data collection, information analysis, and communication. Within the scientific approach, the information gathering stage is a process aimed at addressing the posed questions (Laelandi et al., 2023). One way for students to gather information is to plan and carry out experiments or practicum activities. Scientific education encourages students to observe phenomena that are familiar to them (Narut & Supardi, 2019). One topic closely related to everyday life is the separation of mixtures (Lestari, 2017). One type of mixture separation is chromatography.

The concept of chromatographic separation is of significant importance in introductory laboratories for students, and it is consequently frequently incorporated as a laboratory subject. Experiments involving paper chromatography are commonly featured in introductory laboratory courses, serving as a means to actively involve students in comprehending principles related to polarity, along with fundamental chromatographic concepts (Samarasekara et al., 2018). Chromatography holds a central position in chemistry curricula across various educational levels, spanning from high school to university in numerous countries. Widely regarded as a crucial technique in analytical chemistry, chromatography finds application in nearly every domain of the biological and physical sciences. Its versatility extends to diverse fields, including but not limited to food, pharmaceuticals, industry, health and safety, and forensics (Stankus et al., 2019).

However, at the level of junior high school students tend to understand the basic principle of chromatography according to curriculum 2013 basic competence 3.3: Understanding the concepts of mixtures and pure substances (elements and compounds), physical and chemical properties, physical and chemical changes in everyday life, and basic competence 4.3: presenting the results of investigations or works on the properties of solutions, physical changes, and chemical changes, or the separation of mixtures. Therefore, in junior high school science education, students are required to understand concepts and present the results of investigative work. In secondary education, learning about chromatography is usually done through practical experiments or demonstrations of separating ink on paper using water or solvents (Xie et al., 2020). However, the material on mixture separation is still challenging for students to grasp; students still do not fully understand the concept of chromatography (Lestari, 2017).

To facilitate students' understanding of chromatography, learning can be implemented through experimental methods. In addition to enhancing conceptual comprehension, experimental learning also increases students' motivation in science learning (Kurnia & Daningsih, 2021; Kristanta, 2021; Safitri & Vedianty, 2024). The introduction of simple practicum activities adapted to school conditions can be a solution to increase students' interest and motivation in learning science (Nisrina & Rokhmat, 2025). At the junior high school level, paper chromatography is commonly demonstrated using ink on paper, as described in previous studies. However, the application of paper chromatography with leaf pigments has not been widely implemented in schools.

Based on the previous explanation, the author aims to provide students with experience through experimental activities involving the separation of leaf pigments by paper chromatography, using a simple method in the teaching material, especially to separate mixtures in line with the learning objective in the Basic Competence Curriculum 2013. In addition to students understanding the basic principles of chromatography, this can also help them identify the types of pigments present in leaves, since most students typically associate leaves only with the color green. This experiment uses a simple and inexpensive paper chromatography technique to introduce chromatographic principles.

## METHOD

This research developed an experiment-based practicum learning method for separating mixture materials, which was implemented with 7th-grade students at a junior high school. The experimental method involves a teaching approach where students engage in hands-on experiments to experience and validate what they have learned personally. In the teaching and learning process using this method, students have the chance to actively participate, undergo a procedure, observe, analyze, demonstrate, and independently draw conclusions about an object, condition, or process (Haniyah et al., 2014).

This practicum is carried out for 2 lesson hours, or about 90 minutes, with four groups of 7-8 students each. The practicum lasted 2 days. At the beginning of the learning process, students are asked to complete a worksheet of questions before experimenting. This is intended to establish students' initial understanding of chromatography. Furthermore, students identify hypotheses for the independent, dependent, and control variables that have been set. Subsequently, students experiment. On the second day, students present their experiment results to each other and compare them with other groups, then fill out questions and discuss the conclusion.

### Research Materials

The materials used in this experiment are *Iresine diffusa* leaf / Juba's bush leaf, Acetone, and Petroleum ether. The leaf samples were collected from the schoolyard environment, as this plant is relatively easy to find there. The red-colored leaves made students curious about whether chlorophyll is present even though the leaves are not green.

### Research Tools

The tools used in this experiment are a pestle and mortar, a glass beaker, a graduated cylinder, plastic wrap, strip chromatography, a stand and clamp, a balance, a pipette, a pencil, a ruler, toothpicks, and a test tube.

**Variables**

This experiment consists of three kinds of variables: independent, dependent, and control. More detailed variable descriptions are in Table 1.

Table 1. Variables of the experiment

| Variables | Details |
|---|---|
| Independent | The comparison of the amount of solvent<br>A = 1 ml aceton: 9 ml petroleum ether<br>B = 2 ml aceton: 8 ml petroleum ether<br>C = 3 ml aceton: 7 ml petroleum ether<br>D = 4 ml aceton: 6 ml petroleum ether |
| Dependent | Rf values for each pigment in the leaf. |
| Control | Type of leaves, type of filter paper (2 x 18 cm), duration of paper chromatography conducted (15 minutes). |

**Procedure**

The first step in this experiment is to prepare the tools and materials to be used. The first step is to extract the leaves of *Iresine diffusa*. Then prepare the solvent to be used as the mobile phase in the paper chromatography experiment, which will serve as the independent variable. Lastly, conduct the paper chromatography experiment to separate the pigment of the *Iresine diffusa* leaf.

In the first procedure, the diffusa leaves are cleaned and separated from their veins, then weighed at 8 grams. Subsequently, they are crushed and mixed with 2 ml of acetone. The addition of acetone aims to dissolve the pigments present in the leaves. The mixture is covered with plastic and left for 10 minutes. The leaf extract is then dropped onto a filter paper using toothpicks, with the filter paper positioned 2 cm from the bottom. Perform this procedure on other filter paper, as the experiment involves four different solvent ratios. This procedure is also described in Figure 1.

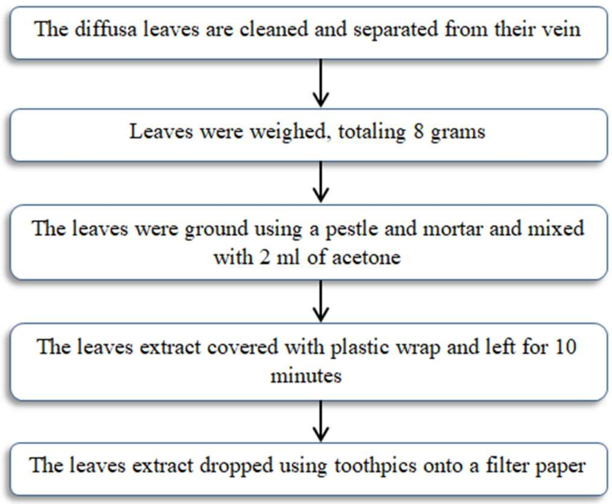



Figure 1. Flowchart of preparation of *Iresine diffusa* leaf extract

While the leaf extract on the filter paper dries, prepare the mobile phase. This procedure is also described in Figure 2. For the first solvent, a ratio of 1:9 of acetone and petroleum ether is used; the second solvent uses a ratio of 2:9, the third uses a ratio of 3:7, and the fourth uses a ratio of 4:6. In this experiment, different solvent ratios were applied to determine the most effective solvent composition for separating leaf pigments through paper chromatography. These variations were designed to modify the polarity of the mobile phase, which directly influences the migration and separation of pigments on the chromatography paper. Acetone is a polar solvent, while petroleum ether is non-polar. Increasing the proportion of acetone increases the solvent mixture's polarity, allowing polar pigments such as chlorophylls to move farther along the paper. Conversely, when the mixture contains a higher proportion of petroleum ether, the solvent becomes less polar, favoring the movement of non-polar pigments such as carotenoids. Thus, varying solvent ratios enable identification of the optimal polarity that yields the best pigment separation. This approach ensures more apparent differentiation and more accurate identification of the various pigments present in the leaf extract. The preparation of these solvents must be done as quickly as possible due to the volatile nature of both solvents.

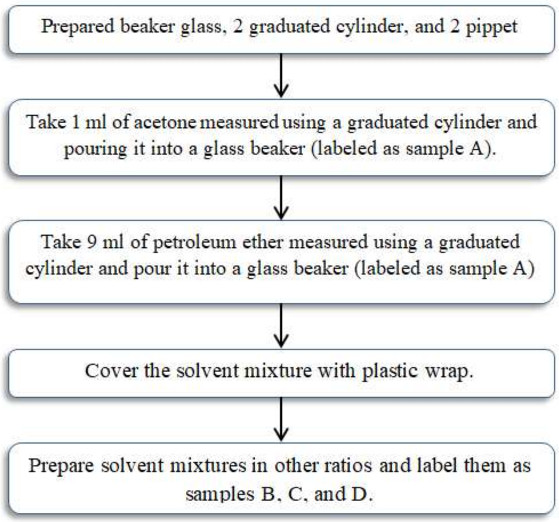



Figure 2. Flowchart of preparation of the solvent as the mobile phase

After the materials are ready, the next step is to perform the paper chromatography procedure. The practicum tool is designed as a test tube mounted on a stand and clamp. 5 ml of solvent is then poured into the test tube and covered with plastic wrap for 2 minutes. This is done to prevent outside air from entering and saturating the gas inside with the mixture's composition. Afterward, the filter paper treated with leaf extract is placed into the test tube. Cover it again with plastic wrap and wait for 15 minutes. This procedure is also described in Figure 3.

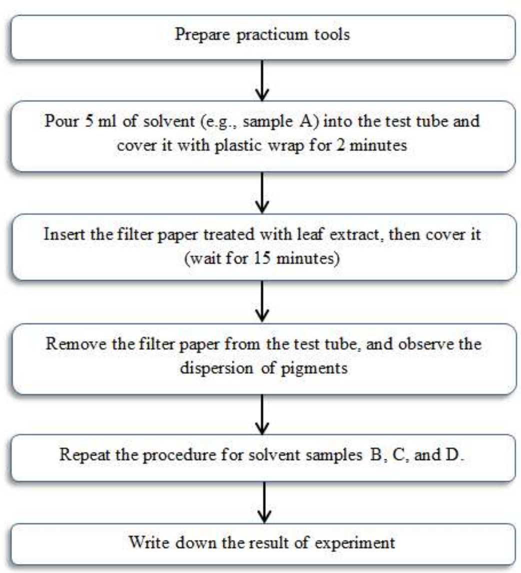


Figure 3. Flowchart of the separation of leaf pigments by paper chromatography

**Student's Response**

The data collection technique employed in this study is the questionnaire method. The questionnaire comprises 10 closed-ended questions. The questions are divided into two categories: (1) practicum significance and (2) learning motivation (Kurnia & Daningsih, 2021). Respondents can choose the statement that best aligns with the given fact. Each statement item provides four response options: Strongly Agree, Agree, Disagree, and Strongly Disagree, covering from positive to negative. Student responses are analyzed using a Likert scale. The percentage value is used to see students' responses toward learning through the practicum. The percentage of students' responses based on (Liana et al., 2023) Categories present in Table 2.

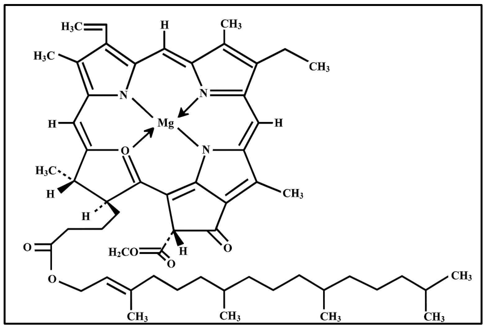
Table 2. Percentage Category of Students' Responses

| Percentage | Categories |
|---|---|
| 80% - 100% | Very High |
| 60% - 80% | High |
| 40% - 60% | Enough |
| 20% - 40% | Low |
| < 20% | Very Low |

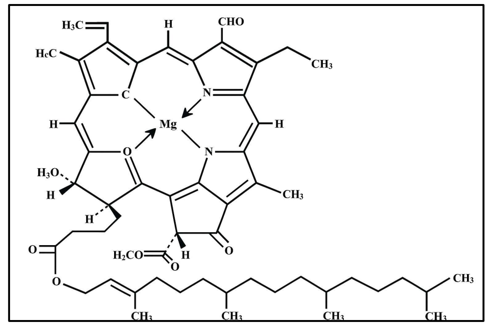
## RESULTS AND DISCUSSION

### Identification of pigment in the *Iresine diffusa* leaf based on the color spot

Leaves contain several pigments, each with a specific role in the process of photosynthesis. The primary pigments found in plant leaves are chlorophyll a, chlorophyll b, and carotenoids. Their chemical structures determine their solubility, making them either polar or nonpolar. (Simkin et al., 2022). Chlorophyll a and chlorophyll b, with chemical formulas $C_{55}H_{72}MgN_4O_5$ and $C_{55}H_{70}MgN_4O_6$, consist of 137 and 136 atoms, respectively. The chemical structure of chlorophyll a and chlorophyll b can be seen in Figure 4.



(A)



(B)

Figure 4. (A) Chemical structure of Chlorophyll-a (B) Chemical structure of Chlorophyll-b

Based on the figure, the difference between Chlorophyll a and Chlorophyll b is that the methyl group on the side chains of Chl a is replaced with an aldehyde group in Chl b. The dipole moment of chlorophyll a is 4.79 – 4.92D, and chlorophyll b is 1.36 – 1.14D. These indicate that chlorophyll a and b are relatively polar (Hedayatifar et al., 2016). These make it soluble in polar solvents. While carotene with chemical formula $C_{40}H_{56}$ is nonpolar, because it is composed of long hydrocarbon chains, making it insoluble in polar solvents but soluble in nonpolar solvents (Alam, 2019; Ciaccio & Hassan, 2020). The solvents used in this study are petroleum ether and acetone. Petroleum ether has an almost 0 D dipole moment, while the dipole moment of acetone is 2.98 D (Barreto et al., 2006). In other words, petroleum ether is nonpolar while acetone is polar. Acetone dissolves polar compounds such as plant-derived compounds or pigments. Meanwhile, nonpolar solvents attract compounds that are completely insoluble in polar solvents. In other words, polar mobile phases facilitate the movement of polar compounds, while nonpolar mobile phases support the migration of nonpolar compounds (Toit et al., 2012). Based on the practical result of paper chromatography, the spread of pigment can be seen in Figure 5.

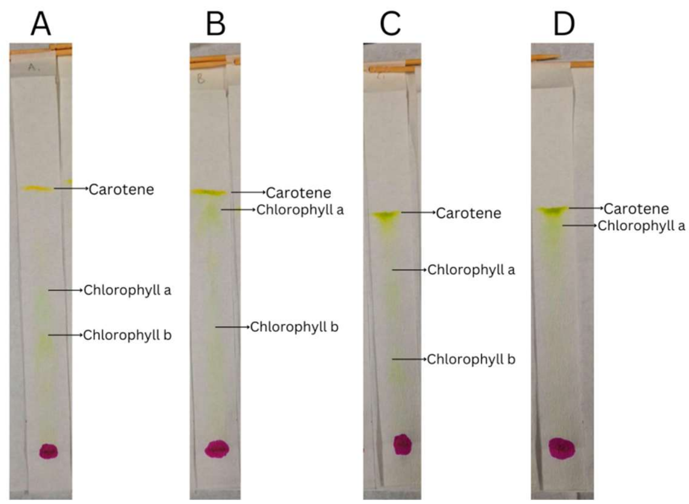



Figure 5. (A) solvent 1:9 (B) solvent 2:8 (C) solvent 3:7 (D) solvent 4:6

Based on Figure 5. In samples A, B, and C, two spots—green and orange — can be identified, whereas in sample D, there is only one green spot and one orange spot. These spots most likely correspond to chlorophyll a and b ( green spot) and b-carotene (yellow-orange) (Toit et al., 2012). Based on the comparison of solvents used, solvent D is more polar than the other three solvents. Highly polar solvents may cause inadequate separation of compounds. This can result in overlapping spots on the chromatogram, making it challenging to distinguish individual components. Also, overly polar solvents may not provide enough interaction with the stationary phase, leading to incomplete separation of the sample components. This can result in broad and poorly defined peaks (Samarasekara et al., 2018). This is similar to the experimental result in which sample D, using a more polar solvent, resulted in incomplete separation of pigment components, as indicated by unidentified color spots, namely chlorophyll b.

From this experiment, students gained a clearer understanding of how paper chromatography separates plant pigments. Students reported gaining a clearer understanding of the working principle of paper chromatography and its application in separating plant pigments. They knew that leaves contain chlorophyll but were unaware that there are different types of pigments, such as chlorophyll a, chlorophyll b, and carotenoids. After performing the chromatography, they identified several pigment bands. Some students also noted that varying solvent ratios led to differences in the distance each pigment traveled. This helped them realize that the polarity of the solvent mixture affects pigment separation. By directly observing the different colors and the varying degrees of separation, they were able to comprehend that the polarity and ratio of solvents play an important role in the effectiveness of the separation process. Comparing different solvents showed that a more polar solvent, such as in sample D, led to incomplete separation and overlapping spots, whereas balanced solvent ratios produced more precise results. This hands-on experience helped students connect theoretical concepts of solvent polarity and solubility with practical outcomes, thereby strengthening their comprehension of chromatography principles. This study is consistent with previous research showing that paper chromatography experiments provide a practical approach to engaging students in learning about polarity and the fundamental principles of chromatography (Samarasekara et al., 2018).

**Identification of Rf value in leaf pigments**

The Retention Factor (Rf) in paper chromatography is a numerical measure of the relative migration of a compound. The Rf value is obtained by comparing the distance traveled by pigments to the distance traveled by the solvent (Kondororik et al., 2015). A higher Rf value suggests that the compound has a greater affinity for the mobile phase (solvent) and has moved a greater distance than other components. Conversely, a lower Rf value indicates less affinity for the mobile phase, resulting in a shorter migration distance. In this experiment, Table 2 provides detailed Rf values for each leaf pigment in different solvents.

Table 2. Rf value in leaf pigments based on different solvents used

| Color Spot | Rf value in solvent (A) | Rf value in solvent (B) | Rf value in solvent (C) | Rf value in solvent (D) | Pigment |
|---|---|---|---|---|---|
| Yellow-green | 0.44 | 0.42 | 0.36 | 0 | Chlorophyll-b |
| Green | 0.60 | 0.91 | 0.83 | 0.91 | Chlorophyll-a |
| Orange | 0.98 | 0.98 | 0.98 | 0.98 | Carotene |

Based on the table, the chlorophyll b pigment shows Rf values ranging from 0.44 to 0.36, which decrease with increasing solvent polarity. Meanwhile, for chlorophyll a, the obtained Rf values range from 0.60 to 0.91, indicating a fluctuating pattern. The highest Rf values are obtained when using solvents B and D. Less polar or, in other words, nonpolar solvents (solvent A) result in a low Rf value of 0.60. Unlike chlorophyll a and b, carotene maintains a constant Rf value, even though different solvent ratios are used. A more detailed explanation of the graph depicting solvent polarity against Rf values for leaf pigments is presented in Figure 6.

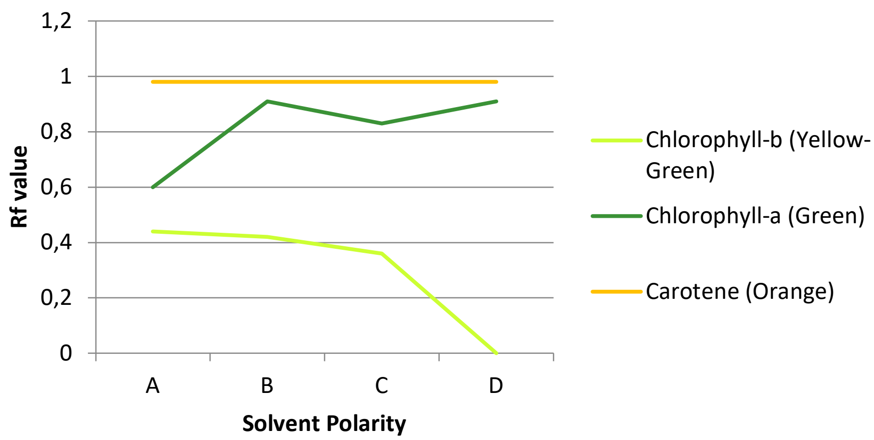



Figure 6. Graph of solvent polarity against Rf values in leaf pigments

Based on the Figure, the Rf value for chlorophyll-b decreases, while that for chlorophyll-a fluctuates, and that for carotene remains constant despite the increasing polarity of the solvent. Solvent D is more polar because the amount of acetone used is greater than that of solvents A, B, and C. The closer the polarity between the sample and the eluent/solvent, the more the sample will be carried by that mobile phase (Elfariyanti, 2022). As explained earlier, the solvents used are both polar and non-polar, and similarly, the pigments present in leaves can be both polar and non-polar. Polar mobile phases facilitate the movement of polar compounds, while nonpolar mobile phases support the migration of nonpolar compounds. (Toit et al., 2012). The constant Rf value for carotene is 0.98. This is consistent with the previous study, indicating that Rf values approaching 0.90 – 1.00 signify the presence of carotene, even though the composition of the solvent used may vary (Elfariyanti, 2022; Paransa et al., 2014).

However, for chlorophyll b pigment, the Rf values tend to decrease, ranging from 0.44 to 0.36. This is also consistent with a previous study on pigment identification, which reported an Rf range of 0.38–0.49 for chlorophyll b. It is noteworthy that in this study, the comparison of the solvents used is different (Kondororik et al., 2015). For chlorophyll a, the Rf values range from 0.60 to 0.91. A previous study also found that the Rf values for chlorophyll a fell within the range of 0.65-0.88, where the solvent used as the mobile phase had a different composition (Arifah et al., 2019). Based on the results, the different solvents used do not affect the differences in RF values. In other words, the polarity of the solvent does not influence the Rf value in leaf pigment, because the range of Rf value remains consistent with other results of research, even though the components of the solvent used are different.

**Students Outcomes**

A total of 29 students completed a questionnaire after the practicum. The students' response questionnaire consists of two subvariables, which were developed into 10 moodified questions. Based on the analysis of the student response questionnaire results on the separation of leaf pigments by paper chromatography, the overall average percentage was 74.43% in the high category. Table 3 shows that the practicum has fulfilled two subvariables: the significance of the practicum and learning motivation

Table 3. Data from Questionnaire Analysis Result of Student Responses to Practicum Activities through Separation of Leaves Pigments by Paper Chromatography

| Sub Variable | Percentage of student response | Category |
|---|---|---|
| Practicum significance | 74.14 | High |
| Learning Motivation | 74.71 | High |
| Average | 74.43 | High |

The first sub-variable is a response questionnaire about the practicum's significance. Student responses in this variable fall into the high category, reaching 74.14%. This indicates that after students conducted experiments to separate leaf pigments using paper chromatography, they found it easier to understand the basic principles and concepts of the technique. Additionally, students became aware that leaf pigments can produce various colors. Indeed, this was a discovery for the students. The student responses to each item of the sub-variable are shown in Figure 7.

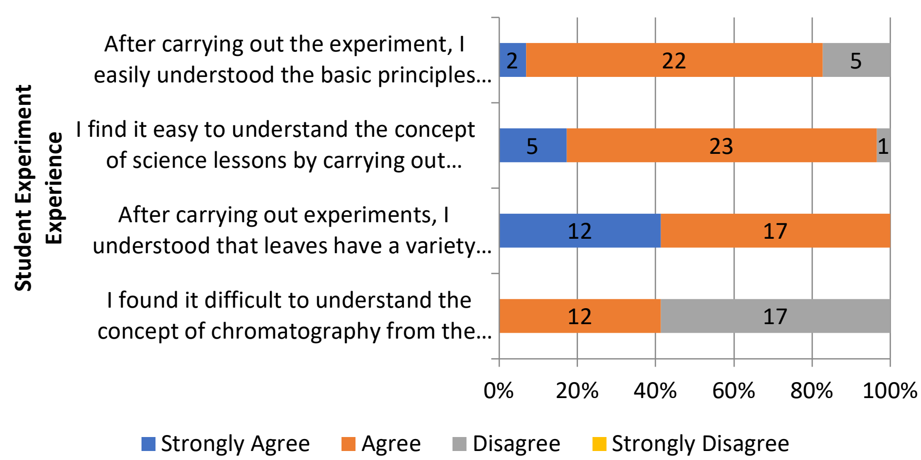



Figure 7. Student responses about the significance of the practicum

Based on Figure 7, students stated that after experimenting, they found it easier to understand the basic principles of paper chromatography. Additionally, students also expressed that they found it easier to grasp the concept after the practical work. These results align with other research findings, indicating that learning activities through practical work can address learning difficulties, such as challenges in understanding the material or difficulties in associating relationships between concepts (Rini & Aldila, 2023). It also states that students can understand scientific concepts through practical activities. In other words, students find it easier to grasp concepts and understand the material when learning is conducted through practical activities (Rahayu & Sari, 2023)

Students also understood that they could identify various types of leaf pigments through this activity. They encountered both difficulties and ease in understanding the concepts of chromatography. More students leaned towards better understanding. However, for students who found difficulty in grasping these concepts, it was mainly due to calculations, specifically the calculation of the Rf value. Some students faced challenges in calculating and determining this Rf value. Engaging in practical methods directly impacts heightened student learning engagement, resulting in improved procedural skills and fostering a more enduring comprehension of materials or concepts (Liana et al., 2023). This occurs because practical activities make the learning process more lively and meaningful (Hayati et al., 2019).

In the second variable regarding student responses to learning motivation, the average percentage is 74.71%, indicating a high category, as shown in Table 2. This shows that the separation of leaf pigments by paper chromatography practicum can motivate students to learn and discover new things. Student responses to each sub-variable item are shown in Figure 8.

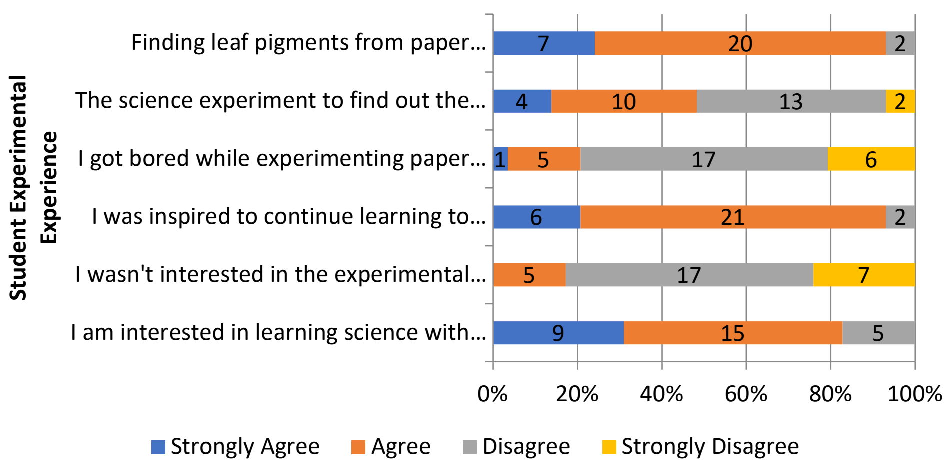



Figure 8. Student responses about the learning motivation

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Based on Figure 8, students believe that discovering leaf pigments through the paper chromatography experiment is an enjoyable lesson. They also express that this experiment inspires them to learn more about science subjects. During the practical activity, students do not feel bored. They also believe that they are inspired to continue learning and express new ideas or concepts after completing this practical activity. Since the experiment is limited to only one type of purple-colored leaf, students are intrigued to try the same activity with leaves of different colors and types. Students are also interested in the experimental method because it helps them understand the concept of paper chromatography. Students state that they are more interested in learning through experiments rather than just listening to lectures. These items are related to learning motivation. Motivation is essential in learning activities (Syar et al., 2023). These results align with other research findings: in addition to understanding concepts more easily through practical activities, students also feel more interested and motivated to learn science. (Rahayu & Sari, 2023). Other research also mentions that the implementation of practical activities makes students more active and motivated to learn science. (Hayati et al., 2019)

## CONCLUSION

Based on the results of the conducted research, it can be concluded that paper chromatography can separate pigments present in leaves. The pigments found in *Iresine diffusa* leaves are chlorophyll a, chlorophyll b, and carotene. However, the polarity of the solvent does not influence the Rf value in leaf pigments, as the range of Rf values remains consistent with other research results, even though the components of the solvent used are different. Students' responses regarding comprehension of the material and motivation to learn during this practical activity also show high levels, with an average of 74.43%. This suggests that implementing paper chromatography not only enhances conceptual understanding but also increases students' engagement and curiosity in scientific investigation. The results of this study can serve as a reference for integrating chromatography experiments into classroom learning, particularly in topics related to mixture separation and photosynthetic pigments. For future development, further research could explore the use of different leaves/plants, alternative solvents, or digital tools to more accurately analyze Rf values.

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