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# Obtaining vegetable dyes as a didactic resource in Biology laboratories

## *Obtención de Colorantes Vegetales como Recurso Didáctico en los Laboratorios de Biología*

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## Abstract

Natural dyes are millenary discoveries that have been used since ancient Greece to color numerous objects such as sculptures but have lost their usefulness and have been replaced by artificial dyes that can be harmful to health. The purpose of this study is to obtain a natural vegetable dye from the hibiscus flower using vinegar as a solvent, to be used as a didactic resource in the experimental practices of the biology laboratory. The hypothesis of the study indicates that a useful colorant can be obtained from the calyxes of the hibiscus flower, demonstrating its function in the experimental observations in the practices of mitosis and meiosis. The method used was descriptive and observational since experimental activities were carried out applying the reagent and dye obtained. The dyes and reagents required for such practices are of high cost and those obtained as a result of this research were of low cost, the acetic orcein in the market has a value of $31.3 for 60 ml, while in the research carried out the cost of the pigment was $0.76 for 60 ml and the reagent that replaced the hydrochloric acid that in the market costs $6 for 500 ml, the reagent obtained had a value of $2.36 for 500 ml; the products obtained had a similar performance to those acquired in the industry. The results contribute with resources that will allow for better.

## Keywords

Learning, dyes, experimental practices, didactic resources, vinegar.

## Resumen

Los colorantes naturales son descubrimientos milenarios que se utilizaban desde la Grecia antigua para dar color a numerosos objetos como las esculturas, pero que han perdido su utilidad siendo sustituidos por los colorantes artificiales que pueden resultar dañinos para la salud. El propósito de este estudio es obtener un colorante vegetal natural a partir de la flor de jamaica empleando como solvente el vinagre, para utilizarlo como recurso didáctico en las prácticas experimentales del laboratorio de Biología. La hipótesis del estudio indica que se puede obtener un colorante útil de los cálices de la flor de jamaica demostrando su funcionamiento en las observaciones experimentales en las prácticas de mitosis y meiosis. El método empleado fue descriptivo y de observación, ya que se realizaron actividades experimentales aplicando el reactivo y el colorante obtenidos. Los colorantes y reactivos que se requieren para dichas prácticas son de alto costo y, los que se obtuvieron como resultado de esta investigación fueron de bajo costo, la orceína acética en el mercado tiene el valor de $31,3 los 60 ml, mientras que en la investigación realizada el costo del pigmento fue $0.76 los 60 ml y el reactivo que reemplazó al ácido clorhídrico que en el mercado cuesta $6 los 500 ml, el reactivo obtenido tuvo un valor de $2.36 los 500 ml; los productos obtenidos tuvieron un funcionamiento similar a los que se adquieren en la industria. Los resultados aportan con recursos que permitirán mejorar la calidad de la enseñanza–aprendizaje en el laboratorio de Biología.

## Palabras clave

Aprendizaje, colorantes, prácticas experimentales, recursos didácticos, vinagre.

## 1. Introduction

Obtaining dyes from vegetables is one of the activities that were performed for many centuries as evidenced by the objects found in cultures since Greece. According to Yusuf, Shabbir, and Faqeer (2017).

> nature has always dominated the synthetic or artificial, since the beginning of this world since nature was the only option for humans to acquire elements, materials of natural origin present characteristics that are advantageous over synthetics, giving them priority (p. 124).

"New technologies are allowing the obtaining of pigments from natural dyes, making them efficient, causing synthetic pigments to be rejected" (Cuesta, 2018, p. 1). The high cost of dyes in the industry where, the value of acetic orcein which is a pigment to give color to chromatin reaches $31.3 the 60 ml in the chemical industry. The scientific experience allows the student to give answers to questions, strengthening their meaningful, lasting and practical learning, enriching their scientific knowledge, in the certainty of being able to live in this world without destroying it and keeping it in good condition for future generations. "Constructivism involves the student to be the constructor and protagonist of his own learning, who brings his knowledge that serves as a support to strengthen the new knowledge" (Miranda-Núñez, 2022, p. 81).

Experimental activities have been considered as a methodological and didactic instrument that supports the academic-scientific training process of students. Teachers who teach subjects in the area of natural sciences frequently incorporate laboratory practices based on a pedagogical model, adapting them to the peculiarities of the environment, to the usable inputs, and to the training needs, since, in addition to supporting the theoretical classes, they awaken and develop the curiosity of the students, motivating them to solve problems (Zorrilla et al., 2022).

The purpose of the research is the extraction of the dye from the calyxes of the hibiscus flower using vinegar as a solvent to substitute acetic orcein, which is commonly used in laboratory practices for the observation of the phases of mitosis and meiosis (chromatin); likewise, lemon and vinegar will substitute hydrochloric acid in the softening of the plant tissues of the samples used in these practices. This dye can be used in Biology laboratories as a didactic resource for the realization of experimental practices, considering that the experiences obtained in the laboratory are fundamental in the teaching-learning process. This will allow the participation and development of students as active beings who construct their own knowledge, obtaining a scientific experience, since they will develop investigative thinking for the understanding of natural phenomena. This knowledge and information can have a new application in the field of formal education and could contribute to the creation of alternative didactic tools to improve the educational process of biological sciences. Hence, it has become mandatory to look for alternatives to obtain dyes by experimental methods such as the one presented in this research, where through the calyxes of hibiscus flowers, the dye was obtained at a cost of $0.76 for 60 ml of the dye.

In this sense, it is necessary to stimulate the biological conditions that allow human beings to construct knowledge. Science teaching is carried out based on pedagogical practices in an interaction that focuses on teaching and learning the contents of the scientific discipline. This space requires a constructivist and contextualized educational practice. The knowledge based on the production of vegetable dyes constitute constructs in the biological sciences, the teacher must deepen in the inquiry which will allow the student to reflect and conceptualize using knowledge and knowledge derived from nature.

Regarding the structure of the article, section 1 contains the introduction in which the reader is familiarized with the context of the work. Section 2 presents the theoretical reference where the documented information on the research topic is gathered. Section 3 details the methods and instruments used to develop this research. In section 4, the results obtained as a result of the study carried out in a theoretical and practical manner are detailed. Section 5 shows the discussion in which the results are extrapolated, interpreted and summarized. In section 6, conclusions are drawn according to the results obtained.

## 2. Theoretical reference

It is possible that the taste for color in human beings is the result of the change of the seasons of the year, and has been shaped by the use of pigments obtained from plants and flowers and even blood and ashes. The accelerated development according to Dupey, and the flourishing of craftsmanship supported mainly by the establishment of Mexican territories, provoked and stimulated the leap from basic dyes (made with earth) to a wider and wider range of dyes. For this reason there was a variety of resources with which colorations applicable in pottery, fabrics, ideograms, rituals, food, and even in the bodies of kings, priests and warriors were obtained (Dupey, 2016). In this regard, Villaño et al. (2016) mention that "the origin of dyes and their classification are due to their biological properties and based on the biological characteristics that dyes possess, the features of their physical properties to their chemical structures are evident" (p. 1).

Thus, according to Valenzuela and Pérez, a variety of colors can be obtained using vegetables, fruits and spices that have diverse applications, since they can be used to dye fabrics, paint wood, color canvases, color homemade soaps, among others. Plant pigments include a wide variety of ranges of components and colors, among the most important we can mention flavonoids, anthocyanins, carotenoids, betalains, chlorophylls among others (Valenzuela and Perez, 2016). "Since the appearance of synthetic dyes in the industry, natural pigments have been relegated, since the synthesized ones present greater stability, wide range of colors and lower cost" (Rolón, 2018, p. 11).

Amchova et al. (2015) state that "in the industry, a series of tests are carried out for the use of different colorants in order to detect the different forms of toxicity" (p. 2). "Natural type colorants are obtained from foods such as fruits and vegetables with intense color and through maceration or boiling processes the colorant can be extracted" (Ulloa, 2017, p. 3). The following table shows the types of colorants with characteristics and examples.

| Natural | Artificial | Vegetables |
|---|---|---|
| Animals: hematoxylin | Synthetics: obtained by fractional distillation of the footprint: Gentian violet | Tornasol, saffron. |
| **By its constitution or dye affinity** | | |
| Direct or substantive: methylene blue and hematoxylin. | Indirect: ferric hematoxylin. | Indirect reversible: indirect hematoxylin. |
| Dyes that require a chemical reaction or development: periodic Schiff's acid. | Dispersed: sudans | Metachromatics: toluidine blue. |
| Reagents: allow superior durability and homogeneity. | Hybrids: higher resistance to degradation by radiation at temperature. | By chemical affinity: blue generated by epifluorescence. |
| **By degree of acidity** | | |
| Acids: sulfonium and carboxyl groups | Basic: contain amino groups with organic groups to form a salt. | |

Table 1. Dye classes Source: (Garrido, 2021).

Thus, "There is little knowledge of the properties of natural colorants" (Ulloa, 2017, p. 2). According to Galarza (2013)

> the production of synthetic pigments is no longer as convenient for producers and buyers, as costs are high, and they are perceived by the public as hazardous to health and the environment, and products containing them are consumed in increasingly smaller quantities (p. 7).

In the words of Jácome et al. (2023) currently, there is "a great interest in natural additives, especially colorants, because these natural compounds have no associated side effects and most of them are functional ingredients, acting as health promoters" (pp.1477-1478). Due to research that has been advanced in reference to the toxicity of synthetic pigments "the interest in natural colorants is growing daily as a result of the continuous eliminations of artificial colorants" (Marcano, 2018, p. 8). Nowadays, people pay more attention to the ingredients in their food and the resources they use on a daily basis.

Hibiscus flowers also called Guinea sorrel, Obelisk, Rosamorada or Karkadé belongs to the Malvaceae family and its scientific name is Hibiscus sabdariffa (Table 2), it is native to Africa where it is considered a very important plant both in its medicinal and nutritional properties, it can reach up to 3 meters in height and its reproduction is carried out by selffertilization. The hibiscus "is a source of bioactive compounds such as polyphenols, flavonoids, ascorbic acid, among others; this composition gives it antioxidant activity" (Sumaya et al., 2014, p. 2).

| **Realm** | **Plantae** |
|---|---|
| Sub-realm | Tracheobionta |
| Division | Magnoliophyta |
| Type | Magnoliopsida |
| Sub-type | Dieeniidae |
| Hierarchy | Malvales |
| Family | Malvaceae |
| Subfamily | Malvoideae |
| Genre | Hibiscus |
| Species | *Hibiscus sabdariffa L.* |
| Common name | Jamaica |

Table 2. Taxonomic characterization of hibiscus Source: (Moposa, 2019).
)

According to Sumaya (2014) "the composition of hibiscus exerts pharmacological effects that produce therapeutic functions in the body that are beneficial to health" (p. 3). According to Pantoja (2022) "hibiscus has different uses, as a medicinal plant to lower cholesterol, triglycerides, decreases body weight, stimulates the functioning of the liver and kidneys and contributes in the absorption of certain minerals." (p. 27). Sumaya (2014) states that

> the cultivation of hibiscus is widespread in Mexico, Central and South America and Southeast Asia, its cultivation and production is expensive because it is often damaged by excessive rainfall, drought or pests, and its harvest requires a high amount of labor to avoid contamination and loss of safety (p. 2).).

It is also known to possess phytochemical compounds such as flavonoids, phenolics, b-carotene, polysaccharides and ascorbic acid. Cruz-Moreno et al. point out that the persistent red color in its calyxes is what gives flavor and color to infusions and prepared beverages, this is due to the content of anthocyanins and the acid flavor to the content of organic acids such as malic, citric, hibiscus and tartaric acid (Cruz-Moreno et al., 2020). On the other hand, flowers contain several natural antioxidant compounds that also act against various viruses and bacteria. One of them "is hisbiscus acid and its derivatives, chemical compounds with antimicrobial properties were identified in them" (Portillo-Torres et al., 2019, p. 2). In the calyxes are found: anthocyanins 1.5 percent, organic acids 15-30 percent, mucilaginous polysaccharides 50 percent, flavonoids, saponins, phytosterols, pectin and fiber. The organic acids and anthocyanins have been shown to have antimicrobial and antioxidant activity. All these components have good bioavailability and have shown therapeutic potential. Other phytochemical components that calyxes possess "are composed of elements rich in anthocyanins, phenolic acids, flavonoids and organic acids." (Izquierdo-Vega, et al., 2020, p. 3).

Jamaica, according to Urbina is an annual herbaceous plant, Malvaceae family that often reaches 1 to 2 meters in height, the stem, leaf petiole and calyxes have a dark or light red color with a tendency to purple or lilac; the flowers are usually born solitary in the axils of the leaves with yellowish petals, and red calyx that usually take one to two days to fall then appear the apices and the fruit or capsule of 5 compartments. The mature plant enveloped by the fleshy calyx is ovoid in shape and contains numerous reniform seeds, pubescent with reddish thread, which take 3 to 4 weeks to develop (Urbina, 2009). Figure 1 summarizes the process of the Jamaican flower.

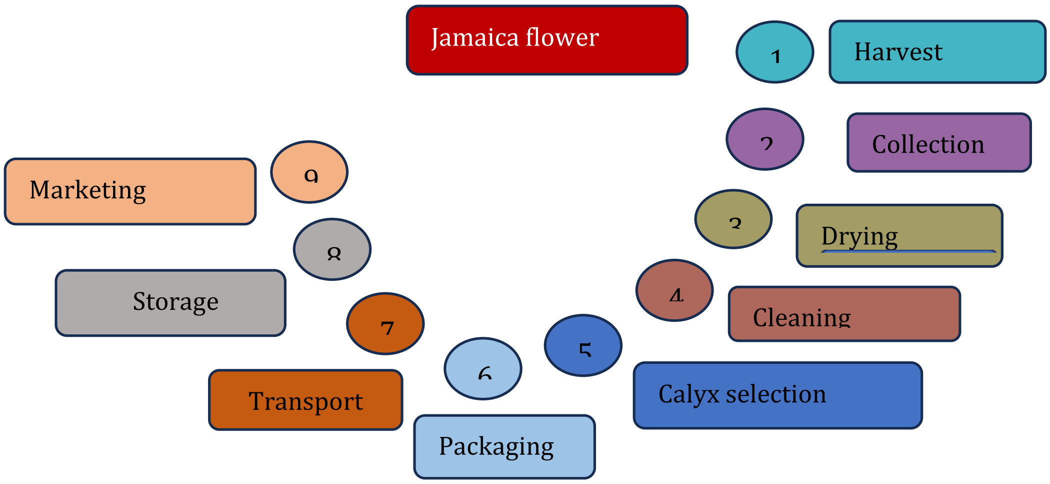


Figure 1. Processing of Jamaica

The process for the commercialization of the hibiscus flower has nine stages (Figure 1). "At the time of ripening of the first calyxes and capsules, the plant is cut from the root, then the calyxes are separated and promptly taken to dehydration" (Rosado, 2020, p. 40). According to Galarza, there are several techniques for the separation of substances such as extraction that allows the organic product to be separated from a reaction mixture or to be isolated from its natural sources, observing the formation of two phases so that the process can be carried out: in the first case a solid and a liquid phase, while in the second case there are two immiscible liquid phases (Galarza, 2013).

There is a diversity of plants that "present special characteristics in their structure that generate pigments such as the red Geranium (Pelargonium hortorum) that has a pale green center contrasting with the margin and flowers arranged in umbels of red, violet pink or white colors" (Estrada, 2021, p. 6). "As for the Red Dahlia (Dahlia pinnata), the expansion of a number of types, colors and sizes offers a solid possibility to the increase of its production" (Jimenez, 2015, p.). "Beetroot (beta vulgaris) is grown mainly for its juice and nutritional value and its roots are blood red with thin skin" (Lopez et al., 2019, p. 368). "Red bell pepper (Capsicum annuum) its odor and color make it attractive for consumption, come from its biochemical components: flavonoids, phenols, epicatechin, rutin, β-carotene, capsanthin, lutein, resveratrol and acids: gallic, chlorogenic and ascorbic" (Martinez et al., 2016, p. 38). Knowing the properties of plants helps the development of experimental practices. Hence, according to López and Tamayo, they are fundamental in the teaching and learning process of science, since, by relating theory with practice, the development of skills and abilities is promoted, the promotion of reasoning abilities, critical and creative thinking providing the student with the construction of knowledge and the strengthening of science with society and culture (López and Tamayo, 2012). Directing the educational process of Natural Sciences in function of satisfying the expressed demands "involves thinking and reasoning, as he observes and experiences the different situations, which allow him to act and establish his own theoretical understanding of the surrounding reality and everything related to it" (Paladines-Condoy et al., 2018, p. 59).

Knowledge management is increasing so it is important to address issues related to innovation and updating of the educational system. Precisely, education must "establish methodological strategies for the development of the class, with the enormous purpose of obtaining positive results for the improvement of teaching in its scientific development" (Calero, 2019, p. 10). Biology laboratory practices should be "encouraged in students by applying simple steps and feasible and easily available domestic resources to strengthen creativity" (Susantini et al., 2017, p. 216). In the Natural Sciences teacher should "prevail the need to be trained to be the student's guide to be a more independent being in the search and assimilation of scientific knowledge through experimentation" (Ramirez, 2023, p. 6).

According to Castellanos (2017) "teachers seek different skills oriented to teaching not only theory, but also towards the convergence of theory and practice, so that there is true meaningful learning" (p. 235). In the same sense, Gil-Álvarez et al. (2017) point out that "the critical-social paradigm, considers the dialectical unity of the theoretical and the practical, as an inseparable whole" (p. 74). It has become visible that stimulation and imagination play an important role in learning and understanding science (Gómez, Ortega, & Lafaid, 2017; González & Palomeque, 2017).

For Reyes (2020) the laboratory is "a great opportunity where the student verifies theoretical concepts that constitute the first contact with the reality that the future professional will face" (p. 63). The use of experimental practices from the constructivist framework "strengthens knowledge and achieves the development of scientific competencies by promoting student participation, so that they are the ones who propose and execute practices that address conceptual, procedural and attitudinal dimensions" (Espinoza-Ríos et al., 2015).

For this reason, it is necessary to stimulate the biological conditions that allow human beings to construct knowledge. Science teaching is carried out based on pedagogical practices in an interaction that focuses on the teaching and learning of contents specific to the scientific discipline. This requires a constructivist and contextualized educational practice. The knowledge based on the production of vegetable dyes constitutes constructs in the Biological Sciences; the teacher must deepen in the inquiry, which will allow the student to reflect and conceptualize using knowledge and knowledge derived from nature. Coronado and Arteta indicate that students are expected to strengthen some skills, for example, seek or formulate reasons to phenomena or problems, create logical and propositional arguments of what is perceived, explain equal phenomena using conceptual characters appropriate to different degrees of complexity, establish cause-effect relationships, combine ideas in the construction of texts, employ mathematical ideas and techniques (Coronado and Arteta, 2015, p. 9). For this, didactic resources that are fundamental in the development of laboratory practices in Biological Sciences are required, such as dyes, since they have been able to make organic matter visible and to identify the cellular structure that allows life to exist.

## 3. Methods and instruments

En la investigación se aplicó el método descriptivo y de observación, ya que se realizaron prácticas experimentales, aplicando el reactivo y colorante obtenidos, mismos que permitieron la visualización de las diferentes fases que conforman la meiosis y mitosis. Dichas prácticas fueron realizadas adaptando los protocolos de Andrade et al. 2005 en los que se observa células sexuales y somáticas vegetales.

### 3.1 Material and Methodology

The material selected to replace acetic orcein which in the market reaches a price of $31.3 per 60 ml, was hibiscus flower which produces a very strong dye used in the manufacture of textiles (Arrascue, 2018), and its cost is $0.84 per 30g and vinegar was used as solvent which has a cost of $0.86 per 500 ml. Therefore, the total cost of dye obtained in the research (acetic soda[1]) is $0.76 per 60 ml. On the other hand, the reagent (hydrochloric acid) with which the softening of the vegetable tissues is carried out has a cost in the chemical industry of $6 for 500 ml, being replaced by lemon juice which has a value of $2.75 for 700 g and vinegar at 5%, thus the definitive value of the reagent that replaced the hydrochloric acid (Auran acetic acid[2]) is $2.36 for 500 ml.

### 3.2 Obtaining the dye

#### 3.2.1 Phase 1 test:

As shown in Table 3, prior to the final standardization of the dye, different plant tissues possessing a red color were tested, thus 20 ml of 5% vinegar was diluted with 6 g of red geranium Elargonium hortorum, 20 ml of 5% vinegar with 2. 3 g of red dahlia Dahlia pinnata, 50 ml of 5% vinegar with 40 g of beet Beta vulgaris and 20 g of red bell pepper Capsicum annuum with 20 ml of 5% vinegar and did not obtain a pigment that allows to clearly color the chromosomes in the nucleus.

#### 3.2.2 Phase 2 preparation of plant extract

The calyxes of hibiscus flowers available in the city's markets were acquired and those in good condition were selected. Then, to extract the dye, 6 g of calyxes were subjected to two tests, one with 70% alcohol and the other with 5% vinegar, which were used as solvents, leaving them to stand at room temperature for 48 hours in an amber-colored flask. Then, the dye was filtered and it was observed that the vinegar extracted the pigment from the calyxes with a higher concentration.

#### 3.2.3 Sample Standardization Phase 3

Different amounts of 5% vinegar were tested with different grams of Jamaican flower calyxes (Table 4), showing that 6 g of calyxes and 20 ml of 5% vinegar resulted in an optimum violet color for coloring the chromosomes in mitosis and meiosis. This substance

[1] Sabda acetic acid is the name given to the dye obtained as a result of this project that replaces acetic orcein in chromatin observation.

[2] Auran acetic is the name given to the reagent obtained as a result of this research that replaces hydrochloric acid in the softening of plant tissues.

was left to stand at room temperature for 48 hours in an amber-colored flask, after which the dye was filtered to remove organic residues.

## 3.3 Obtaining the reagent

### 3.3.1 Quantity and time modification Phase 1

1 ml of lemon juice was extracted and mixed with 1.5 ml of 5% vinegar, this reagent was added to the root apices of Allium cepa and the anthers of Lilium candidum, placing these elements on different watch glasses for 10 minutes, no flaming was used in this phase and there was no softening of plant tissue (see Table 5).

### 3.3.2 Phase 2 quantity and time modification 2

1.5 ml of lemon juice was extracted and mixed with 1.5 ml of 5% vinegar, this reagent was placed on the root apices of Allium cepa placed on a watch glass for 18 minutes. The same process was carried out with the anthers of Lilium candidum. No flaming was used and there was no softening of plant tissues (see Table 5).

### 3.3.3 Pre-standardization Phase 3

1.5 ml of lemon juice was extracted and mixed with 1 ml of 5% vinegar, this mixture was placed on the root apices of Allium cepa placed on a watch glass, 2 intervals of flaming for 1 minute and 2 intervals of rest of 1 minute were performed, similar process was performed with the anthers of Lilium candidum, only the external tissue was softened (see table 5).

### 3.3.4 Phase 4 reagent standardization

In 1 ml of lemon juice and 1 ml of 5% vinegar, this reagent was placed on the root apices of Allium cepa located on a watch glass where 4 intervals of flaming for 1 minute and 4 intervals of rest of 1 minute were carried out, with the anthers of Lilium candidum the same steps were followed. There was total softening of the root tissue and anthers (see Table 5).

# 4. Results

In graphs 2, 3, 4 and 5 it is appreciated that the obtained colorant (Sabda acetica) colors in a similar way to acetic orcein, the obtaining of this pigment was made through the dissolution of 6 g of calyxes of the hibiscus flower in 20 ml of vinegar at 5% (see table 4) and to replace the hydrochloric acid that allows the softening of the vegetable tissues 1 ml of lemon juice was used with 1 ml of vinegar at 5% (Auran acetica) for a total of 8 minutes. By combining the flaming (4 intervals of 1 minute) with the resting time (4 intervals of 1 minute), which should be interspersed with 1 minute of flaming and 1 minute of resting to complete the 8 minutes established for softening (see Table 5). This process allowed coloring and softening the plant tissues in a similar way to the procedure developed with acetic orcein and hydrochloric acid in the observation of the phases of mitosis and meiosis (graphs 2, 3, 4 and 5).

| Species | Grams of the species | 5% vinegar |
|---|---|---|
| **Red geranium**<br>*Elargonium hortorum* | 6 grams | 20 ml |
| **Red Dahlia**<br>*Dahlia pinnata* | 2.5 grams | 20 ml |
| **Beets**<br>*Beta vulgaris* | 40 grams | 50 ml |

| | | |
|---|---|---|
| **Red bell pepper** *Capsicum annuum* | 20 grams | 20 ml |

Table 3. Obtaining the material

Table 3 shows the experimental procedure describing how the optimum biological material was chosen for the elaboration of the dye, which was obtained by relating the weight of the different flowers of the various species with different ml of 5% vinegar.

| Jamaica | 5% Vinager | Color |
|---|---|---|
| 3 grams | 35 ml | Pigmented red |
| 4 grams | 35 ml | Munsell Red |
| 4 grams | 30 ml | Pantone Red |
| 5 grams | 25 ml | Crimson Red |
| 6 grams | 20 ml | Violet red |

Table 4. Dye concentration standardization process.

Table 4 shows the experimental procedure describing the standardization of the colorant, which was obtained by relating the weight of hibiscus to the ml of 5% vinegar.

| Test of experimentation | Lemon | 5% vinegar | Flamed | Resting time | Result |
|---|---|---|---|---|---|
| 1 | 1 ml | 1.5 ml | 0 minutes | 10 minutes | No flaming was used. No tissue softening. |
| 2 | 1.5ml | 1.5 ml | 0 minutes | 18 minutes | No flaming was used. No softening of the fabric. |
| 3 | 1.5 ml | 1ml | 2 intervals of 1 minute | 2 intervals of 1 minute | Joined flaming intervals with rest intervals. Softening of external tissue only. |
| 4 | 1 ml | 1ml | 4 intervals of 1 minute | 4 intervals of 1 minute | Joined flaming intervals with rest intervals. Total softening of plant tissue. |

Table 5. Softening process of plant tissue for the observation of meiosis and mitosis.

En el cuadro 5 se aprecia el procedimiento experimental. Allí se describe la estandarización de la substancia que permitió el ablandamiento de las raíces de cebolla *Allium cepa* y anteras de lirio *Lilium candidum* para observar las fases mitosis y meiosis respectivamente.

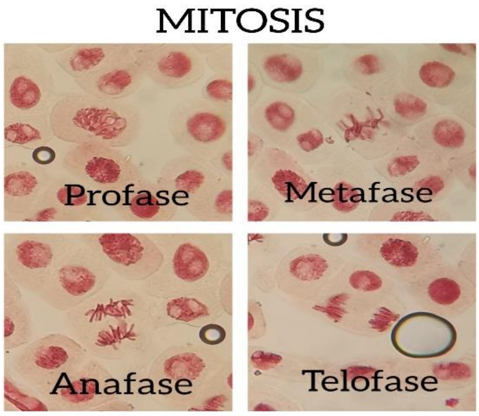


*Figure 2. Application of the dye Sabda acetica for the observation of the phases of mitosis in root tissue of Allium cepa.*

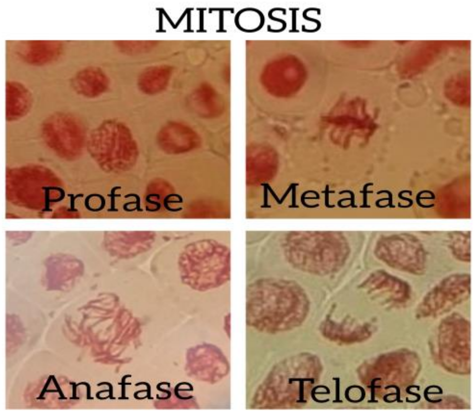


*Figure 3. Application of acetic orcein dye for the observation of mitosis phases in root tissue of Allium cepa.*

Graph 2 clearly shows the mitosis carried out with onion roots (Allium cepa) using the organic dye hibiscus (Sabda acetica) and the softening of the plant tissue with the mixture of lemon juice and vinegar at 5% (Auran acetica), which allowed the visualization of the phases. Figure 3 shows the mitosis using the dye acetic orcein and hydrochloric acid to soften the plant tissue, showing that the dye produced with calyxes of hibiscus flowers pigments in the same way as with acetic orcein and the mixture of lemon juice with vinegar softens the plant tissues as well as hydrochloric acid.

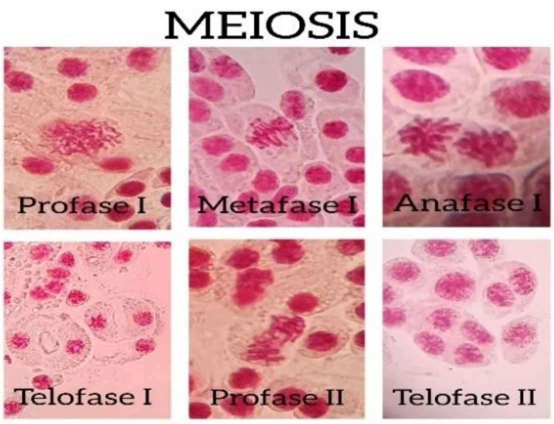


Figure 4. Application of Sabda acetic dye for the observation of meiosis phases in anthers of Lilium candidum.

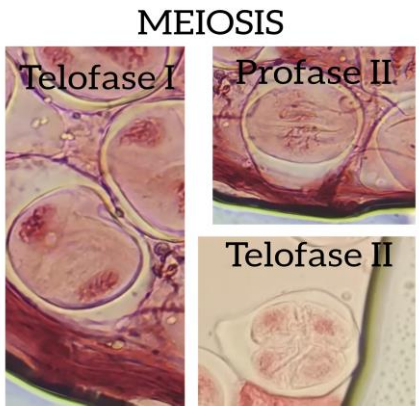


Figure 5. Application of Sabda acetic dye for the observation of meiosis phases in anthers of Lilium candidum.

Figure 4 clearly shows the meiosis carried out with onion lily (Lilium candidum) anthers using the organic dye of hibiscus (Sabda acetica) and the softening of the plant tissue with the mixture of lemon juice and vinegar at 5% (Auran acetica), which allowed the visualization of the phases. Figure 5 shows the meiosis using the dye acetic orcein and hydrochloric acid to soften the plant tissue, demonstrating that the dye produced with calyxes of hibiscus flowers pigments in the same way as with acetic orcein and the mixture of lemon juice with vinegar softens the plant tissues as well as hydrochloric acid.

## 5. Discussion

The dyes found in the chemical industry used for laboratory practices are of high cost, which prevents the execution of experimental practices in educational institutions both at middle and higher levels. Therefore, it is necessary to look for new alternatives for the elaboration of low-cost dyes that are affordable, such as the one developed in this research (Sabda acetica), so that the practical component can be carried out in subjects such as Biology in educational institutions that do not have the necessary economic resources.

Similarly, for the procedure of softening plant tissues, hydrochloric acid is commonly used for experimental practices; however, it was proposed to obtain a substitute that fulfills the same function of this reagent from the mixture of lemon with vinegar (Auran acetic acid), demonstrating that its application has the same function as hydrochloric acid. It should be emphasized that the methods used in the experimental part established the basis for the collection of data and later analysis of the information obtained. At the time of the experimentation, several tests were carried out until reaching an effective standardization, once the softening and staining process was established, it was possible to observe the division of plant cells.

## 6. Conclusions

Plant dyes are indispensable resources that have diverse applications in the industry and as didactic resources in experimental practices in the teaching-learning process of biology; therefore, it is important to strengthen research on obtaining this type of dyes. The study employed a technique that significantly reduces the economic resources that are invested to obtain the inputs that are used to carry out the laboratory practices in plant and animal tissues in which chromatin (chromosomes in mitosis, meiosis, polytene chromosomes and Barr bodies) can be clearly observed.

Generally, for the softening process of plant tissues, reactive hydrochloric acid is required, which is expensive and presents a risk in its handling during experimentation. However, mixing 1 ml of lemon juice plus 1 ml of 5% vinegar for 8 minutes and flaming this compound for 4 minutes minimizes the danger and cost of its use. Acetic orcein is a high-cost dye used in experimental practices to stain chromatin, which makes it necessary to look for alternatives to obtain dyes that fit the budget of educational institutions. In the research carried out after developing several tests, the dye was standardized by diluting 6 g of calyxes of hibiscus flowers with 5% vinegar, which allowed a similar or better visualization of chromatin in plant tissues in comparison with acetic orcein.

The resources used to replace acetic orcein, which in the market reaches a price of $31.3 per 60 ml, were the calyxes of the hibiscus flower that produce a very strong dye and its cost is $0.84 per 30g and vinegar was used as solvent, which has a cost of $0.86 per 500 ml, the total cost of the dye obtained in the research (acetic soda) is $0.76 per 60 ml. On the other hand, the reagent (hydrochloric acid) used to soften the vegetable tissues has a reference value in the chemical industry of $6 per 500 ml, being substituted by lemon juice which has a value of $2.75 per 700 g and vinegar at 5%. The definitive value of the reagent that replaced hydrochloric acid (Auran acetic acid) is $2.36 per 500 ml.

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