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Influence of *Erythrina crista-galli* L. Extract Natural Dye in Plant Histology Staining

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Abstract. The development of the use of preparations with natural coloring has been carried out a lot, but no one shows optimal concentration. The purpose of this study was to determine the optimal concentration of *Erythrina crista-galli* L. flower extract as a natural dye based on the parameters of the quality and stability of the preparation. The type of research used is experimental research. This research method through several stages: extracting, diluting extracts with various concentrations (30%, 50%, 70%, 90%, and 100%), until making maceration preparations. Data collections were conducted twice, where the first and second take has an interval of one semester. Kruskal Wallis was used as a technique for analyzing data from research results. Both in the first and second observations, the concentration of 70% always produces the best quality preparations. However, the stability of natural staining was not good due to color quality was decreasing. Thus, although *Erythrina crista-galli* L. has a good potency, the flower extract has not recommended as natural staining.

INTRODUCTION

At present, many techniques and procedures are used to dye various tissue characteristics and microscopic structures of cells [1]. Significant changes have been made to the methods used for histological staining through chemicals substances [2]. On the other hand, the histological staining technique using natural dyes [3], has also begun to become a new alternative [4].

Nature has always dominated over synthetic or artificial [5], from the beginning of this world as nature was the only option for a human being then, and now with advantageous characteristics of naturally derived materials over synthetics giving them priority [6]. Natural dyes are to be considered as an alternative to the synthetic dyes used today.

Anthocyanins are naturally occurring pigments of red and purple [7]. Red anthocyanin pigments provide stable and sharp and widely applied in various industries [8]. *Erythrina crista-galli* L. flower is an alternative natural coloring agent because it has high anthocyanin content. The previous study informed that in flower extract of the plant, anthocyanins content to reach 85.86% [9]. This finding is in line with information that states that in the petal section, it has large amounts of anthocyanin biosynthetic activity [7]. The utilization of this flower as a source of natural dyes is increasingly potential because anthocyanins are also reported to be easily absorbed by cells [10].

The use of natural dyes in making preparations is not without obstacles. In addition to determining natural herbs [11] and technique [12], various obstacles include; Determination of the correct concentration [13,14], quality, and color stability of preparations [4,15], is often an obstacle. Moreover, the focus of this research is looking at the quality and stability of the staining of the preparations, because in addition to the imaging of apparent objects, color stability significantly affects preparations that use natural ingredients, so that preparations can be used for a long time.

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MATERIALS AND METHODS

Material

The instrument used for the manufacture of *E. crista-galli* L. flower extract; Erlenmeyer, Glass funnel, Beaker glass, Filter cloth, Pumpkin measure, Electric stove, Soxhlet Extruder, Evaporator/destilator, Stirrer, pH meter. A tool used to make *P. betle* L. stem maceration preparations; Knife/razor, Cover glass, Glass object, Hotplate, Petri dish, Binocular microscope, Drop pipette, Camera, Label paper, Analytical scales, The material used to make extracts is flower *E. crista-galli* L., Aquades, Ethanol 96%, 3% citric acid. The ingredients used to make maceration preparations are P. betle L. rods, Aquades, 10% KOH solution, 10% nitric acid, 10% chromic acid, 30% alcoholic solution, 50%, 70%, 80%, 100%, Xylol, Enthelen, Safranin, *E. crista-galli* L. flower extract, with concentrations of 30%, 50%, 80%, 90%, and 100%.

Methods

This research uses experimental research and quantitative approach. The sample in this study was young *P. betle* L. stems, which were divided into six treatment groups consisting of 3 replications. This research method through several stages: extracting, diluting extracts with various concentrations (30%, 50%, 70%, 90%, and 100%), until making maceration preparations.

Extracting

E. crista-galli L. flower extraction, using the maceration method, the flowers were washed clean, *E. crista-galli* L. simplistic, mashed, given 96% ethanol solution + 3% citric acid, the extract is filtered, extract analysis of the effect of pH, type of solvent, concentration, and extraction temperature. Stored in a dark place at room temperature (25^{o}C) for 24 hours, heated for 3 hours using a rotary evaporator until it thickens, and diluted using distilled water, with various concentrations (30%, 50%, 70%, 90%, and 100%).

Maceration preparation

Boil for 3 minutes with a slice of 0.5 cm *P. betle* L. stem into a falcon bottle containing distilled water until soft, then chill. After chilling, remove distilled water with a 10% KOH solution, soak for three minutes. Transfer the ingredients to the watch glass and wash with distilled water and drain. Add ingredients with a mixture of 10% citric acid, 10% chromic acid one drop until soft, then wash again with distilled water. Dripping *E. crista-galli* L. extract dye with various concentrations (30%, 50%, 70%, 90%, and 100%) and safranin coloring for 1-hour wash again with distilled water. Dehydrate using alcohol 30%, 50%, 70%, 80%, 100%, 100%, three minutes each. Alcoholizing, the alcohol mixture: xylol (3:1, 1:1, 1:3), three minutes each. Transfer the material to the slide and then press the *P. betle* L. stem so that the stem lamella and its vascular tissue are separated. Drop pure xylol I for three minutes. Observe the material under the microscope, while dropping pure xylol II on the slide. If the observation is finished, then give ethylene to the glass object and cover it with a glass cover.

Observation stage & Data analysis

Observe the preparations for P. betle L. maceration, which has been treated under a binocular microscope, and then take pictures of the observations on the microscope using a camera. Data collections were conducted twice, where the first and second take has an interval of one semester. Kruskal Wallis is used as a technique for analyzing data from research results.

RESULTS

The use of various concentrations of *E. crista-galli* L. flower extracts as natural dyes for the quality of *P. betle* L. stem maceration preparations. All the results of the first assessment score were taken in February, while the second was taken in July. Include; clarity of preparations, color contrast, and completeness of tissue components, as can be seen in Table 1.

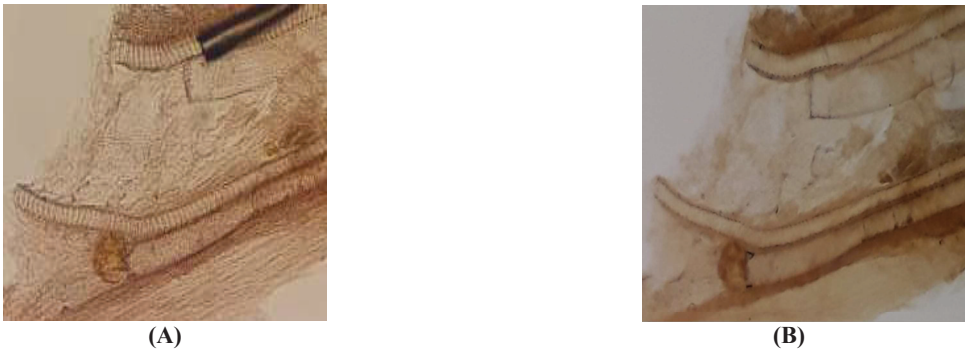
TABLE 1. Recapitulation Comparison of Average Results of Quality of various concentrations of *E. crista-galli* L. flower extracts *P. betle* L. Maceration Preparations in February and July

Treatment	Mean in January	Mean in July
A (30%)	3.45	3.05
B (50%)	4.35	3.96
C (70%)	4,.55	4.13
D (90%)	2,35	1.9
E (100%)	1.85	1.58
P (+)	4.8	4.8

Comparison of results of the stability of natural dyes from flower extraction of E. crista-galli L., using a concentration of 30%, 50%, 70%, 90%, 100%, and Safranin, in February and July, can be seen in Fig. 1-6.

(A) **(B)**

FIGURE 1. Comparison of results the stability of natural dyes from flower extraction of *Erythrina crista-galli* L., using a concentration of 30%, (a) in February and (b) July



(A) **(B)**

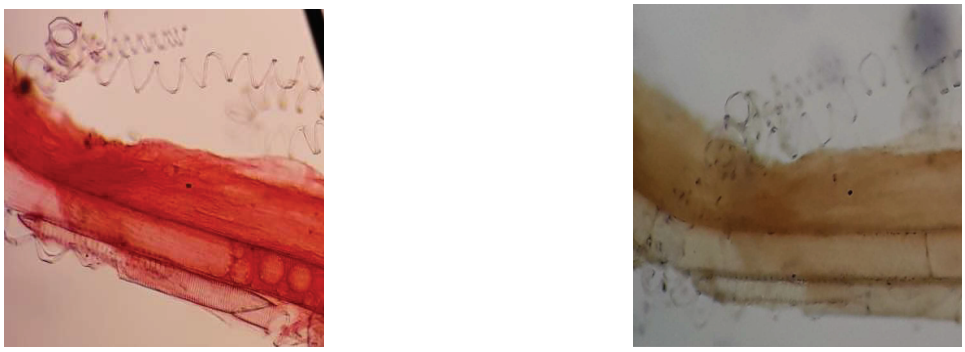
FIGURE 2. Comparison of results the stability of natural dyes from flower extraction of *Erythrina crista-galli* L., using a concentration of 50%, (a) in February and (b) July

(A) (B)

FIGURE 3. Comparison of results the stability of natural dyes from flower extraction of *Erythrina crista-galli* L., using a concentration of 70%, (a) in February and (b) July

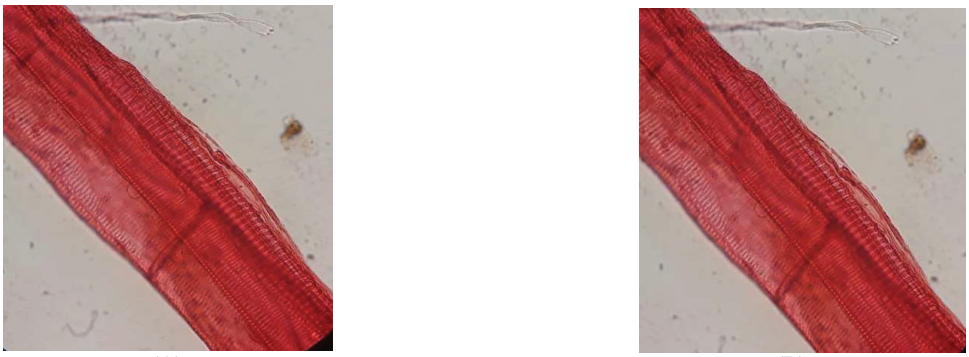
(A) (B)

FIGURE 4. Comparison of results the stability of natural dyes from flower extraction of *Erythrina crista-galli* L., using a concentration of 90%, (a) in February and (b) July



(A) (B)

FIGURE 5. Comparison of results the stability of natural dyes from flower extraction of *Erythrina crista-galli* L., using a concentration of 100%, (a) in February and (b) July



(A) (B)

FIGURE 6. Comparison of results the stability of natural dyes from flower extraction of *Erythrina crista-galli* L., using a safranin, (a) in February and (b) July

DISCUSSION

Based on Table 1, it is demonstrated that the results of each treatment have different qualities. The treatment that had the highest average score was safranin with a mean score of 4.8, without changes from January to July. While the treatment using flower extract *E. crista-galli* L., the highest average score at a concentration of 70%, this can be seen in the initial data and the final data, with an average score of 4.55 in January and a mean score of 4.13 in the month July. This means that there is a change in color quality in the *Piper betle* L., maceration preparation. This proves that the more precise a concentration of extract as coloring plant tissue preparations, it can produce good quality preparations[10].

The results of a comparative study of the quality and color stability of *P. betle* L. stem maceration preparations using *E. crista-galli* L. flowers, Fig. 1-5, do not show clarity of the tracheal vessels consisting of two components making up the tissue, namely the primary wall and thickening of the secondary wall, a dense spiral type, compared with the safranine dye preparation in Fig. 6. This shows the color quality and stability. Evidenced in initial data collection in January and final data collection in July with a span of six months. Color changes occur in preparations that are increasingly fading, so the preparations are not precise, including contrast, and some components are not visible. This is because the absorption of anthocyanin pigments into plant tissue begins to disappear.

The loss of anthocyanin staining can be influenced by the ability of plants to absorb different types of anthocyanin from various types of plants [16], including those produced by *E. crista-galli* L. flower extracts on *P. betle* L. stem preparations. Anthocyanine can be stored in vacuoles of cells in some plant tissues, so to maintain their presence in specific tissues, anthocyanins accumulation varies in the amount [16] and the ability of cells to absorb anthocyanins in plant tissues [17].

The reduced ability of pigment absorption in plant tissue is also affected by sunlight, rehydration temperature, and heating [18], so the pigment also decreases. The storage duration of the preparations is also [19], and a temperature of 15^{o}C can affect the color stability decrease. The anthocyanin stability is always followed by color loss, so it is possible to determine the protective effect of pigments as a solution, because pigmentation is an important factor of anthocyanin stabilization, in plant vacuoles, where the pH approaches 7.0, the intense blue/red colour observed has been associated with anthocyanin complexation. Some copigments can protect the nucleophilic attack of water, which often causes loss of colour [20].

SUMMARY

In this study, the potential of *E. crista-galli* L. extract as a natural dye for *P. betle* L. preparations was analyzed. The results showed that 70% concentration was able to dye preparations of the highest quality. Unfortunately, after the second observation, the color of the preparations deteriorated. Therefore, despite being able to color well, the stability of this plant extract is still not recommended.

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