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## *Curcuma longa* Linn rhizome extract as an alternative stain for histological studies

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### Abstract

Most chemically-synthesized stains used in histological practices are expensive, hazardous to human and animal health, and threatening to the environment. Extraction of natural dye out of local plants have gained global interest and give promising tissue staining when outcomes are investigated. *Curcuma longa* rhizomes are proven to contain curcuminoids that exhibit various medicinal properties, and curcumin the bright yellow component of the plant that is being used as colorant. This natural dye from *C. longa* provides significant alternative as they are safer to use without health hazards, biodegradable, and have easy disposability. Nine biological specimens were used to compare the staining capacity of 15, 20, and 25-gram concentrations of *C. longa* extract and the commercial stain and were observed under microscope. The results revealed that the 15, 20, and 25-gram concentrations of *C. longa* stains do not have significant differences in the staining effect on various plant and animal tissues. Also, the staining effects of commercial and *C. longa* stains on monocot and dicot root and stem, leaf midrib, frog blood smear, and cycloid scale do not have significant differences. Therefore, the alternative stain out of *C. longa* can be used as substitute to commercially available stains.

**Keywords:** Alternative stain, *Curcuma longa* Linn, histology, staining

### Introduction

At the height of technological advancement and mass production of goods, people nowadays would prefer to use natural and organic if given a chance to choose against the commercial, chemically-synthesized products. Why choose natural or alternative products? Aside from reducing if not avoiding skin or body parts irritation when they are used, the natural organic products have stood over the test of time, giving people no side effects. Using natural organic products is environment-friendly aside from the fact that they are more economical.

The turmeric or the *Curcuma longa* Linn is an example of the plant that a natural product can be derived. It is a tropical and sub-tropical perennial herb belonging to the family Zingiberaceae which is related to ginger. The genus *Curcuma* includes many other economically important species, including *C. Angustifolia* (wild arrowroot, narrow-leaved turmeric), *C. Amada* (mango ginger), and *C. zedoaria* (zedoary), and *C. aromatica* (Cochin turmeric, wild turmeric). The exact origin of this plant is not identified, but it is thought to originate from South or Southeast Asia, most probably from China, Vietnam, or western India. India is the world's largest producer, consumer, and exporter of turmeric but it is cultivated widely in Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia and the Philippines. (Chairman *et al.*, 2015) [8].

It is also proven to contain medicinal properties like antidiabetic, anti-ulcer, anti-coagulant, antifertility (Yadav *et al.*, 2017) [22], antiviral, antibacterial, antifungal (Moghadamtousi *et al.*, 2014) [17], antimicrobial (Suebkhampet *et al.*, 2014) [20], antioxidant, anti-inflammatory, antiseptic and anti-cancer. Moreover, rhizomes of *C. longa* are proven to contain curcuminoids that have been demonstrated to be antiatherosclerotic, anti-inflammatory, etc. (Foldesiova *et al.*, 2016) [11]. Additionally, it has also been shown to treat jaundice, flatulence, hemorrhage, hematuria, and skin diseases (Labban, 2014) [16]. This plant plays a vital role in preventing and treating numerous ailments from cancer to neurological, autoimmune, and cardiovascular illnesses (Kocaadam *et al.*, 2015) [12]. Likewise, this is also publicized to have the anti-HIV activity to fight acquired immunodeficiency syndrome (AIDS) (Nasri *et al.*, 2014) [18] to those who are infected with the virus.

Aside from being recognized for its medicinal benefits, *C. longa* is used extensively as traditional Indian spice (Krup *et al.*, 2013) [14], the source of coloring agent for foods, textiles, and cosmetics (Dash *et al.*, 2014) [10]. Its tuberous rhizomes have been used as a condiment, an aromatic stimulant, and colorant. The bright yellow colored compound found in Turmeric is called curcumin. Such main fluorescent component or the curcumin in Turmeric is so

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dominant that it could be used as a stain. In biology laboratories where experiments using microscope would be an exciting part of the student life on the school campus, Turmeric as the alternative stain is interesting.

A stain is a discoloration that can be clearly distinguished from the surface, material, or medium it is found upon. They are generally used to add color to plant and animal tissues, microbe, and spores to make them optically distinct (Korade *et al.*, 2014) [13]. Most stains in current use are synthesized from mixtures of various chemicals. Aside from being expensive (Aguoru *et al.*, 2016) [2], they are also hazardous to human (Braide *et al.*, 2011) [7] and animal health (Suryawanshi *et al.*, 2017) [21]. Likewise, some staining procedures have been abandoned because the chemicals needed have been scientifically proven to be lethal (Alturkistani *et al.*, 2016) [4]. Therefore, natural plant dyes have gained global interest and give promising tissue staining results when tested. In Nigeria, many researchers have extracted some colors from a variety of local plants (Abubakar *et al.*, 2012) [1]. Others have proved gumamela, bougainvillea, alugbati, etc. to stain some fungi, bacteria, and protists.

As synthetic dyes displayed environmental threats, it evoked the desire of researcher to come up with a biological stain made of eco-friendly and biodegradable materials. Besides, as many developing countries cannot afford the expense of synthetic dyes, the use of low-cost, natural colors from plants is observed as the substitute for synthetic dyes. Based on those mentioned above, the staining potentials of curcumin turmeric was examined as a natural dye in the histological application.

Furthermore, natural dye from *Curcuma longa* provides a significant alternative as they are safer to use without health hazards, biodegradable, have easy disposability, and can be used as compost material for farming purposes after the necessary components have been extracted (Suryawanshi *et al.*, 2017) [21].

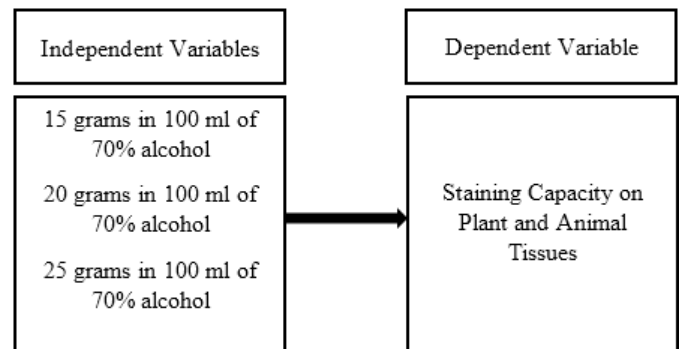
An excellent biological stain must be efficient, inexpensive, less noxious, and the source must be accessible. Almost all stains have specific mechanisms which could be used both in plant and animal tissues (Aguoru *et al.*, 2015) [3]. There are two primary forms of stains based on their origin; synthetic and natural. Synthetic dyes are produced by combining chemicals from petroleum sources (Shehu *et al.*, 2012) [19]. It exhibits excellent fastness properties, available in most varied colors and can be obtained easily. However, most synthetic dyes are expensive (Aguoru *et al.*, 2016) [2], desperate to prepare, allergenic, genotoxic, mutagenic, carcinogenic, immunogenic (Bordoloi *et al.*, 2017) and severely detrimental to the environment that can reduce soil fertility (Korade *et al.*, 2014) [13].

Many researchers have attempted to extract curcumin and used it as a substitute for synthetic biological dyes. The study conducted by Kumar *et al* (2014) [15] revealed that natural tint from turmeric could stain tissues such as collagen and muscle fibers. In the investigations made by Bassey *et al* (2012) [5] and Inbnouf (2017), they concluded that Turmeric has good potential and a promising histological dye that can excellently replace Eosin stain in the Hematoxylin and Eosin routine. On the other hand, Chinyere *et al* (2017) [9] found out that turmeric is not an ideal alternative stain for tissues when compared to Hematoxylin and Eosin stains.

This experimental comparative study aimed to explore the capability of Turmeric (*Curcuma longa* L.) rhizome extracts to replace chemically synthesized, noxious and expensive

histological stain. This research was conducted to discover the right modifications that would enable turmeric to be suitable in replacing synthetic dyes in Histology practices.

### Framework of the study



**Fig 1:** Conceptual Model of the Study

The proposed framework integrates the significant components of the study. The model in Figure 1 presents the relationship between variables of the study. The two variables are the different concentrations of turmeric solutions and its effect on the staining capacity on tissues. This research claimed that different levels (15, 20, 25g) of turmeric dissolved in 100 ml of 70% alcohol would affect staining plant and animal tissues. The researcher utilized the said varying concentrations, considering the results of the study conducted by Kumar *et al* (2014) [15]. Kumar and others prepared 5, 10 and 15 grams concentration but found out that 15 grams of turmeric powder has provided the best result. In the present study, the researcher explored if the increase in the amount of turmeric powder may yield a staining result better than what 15 grams can do.

### Objectives

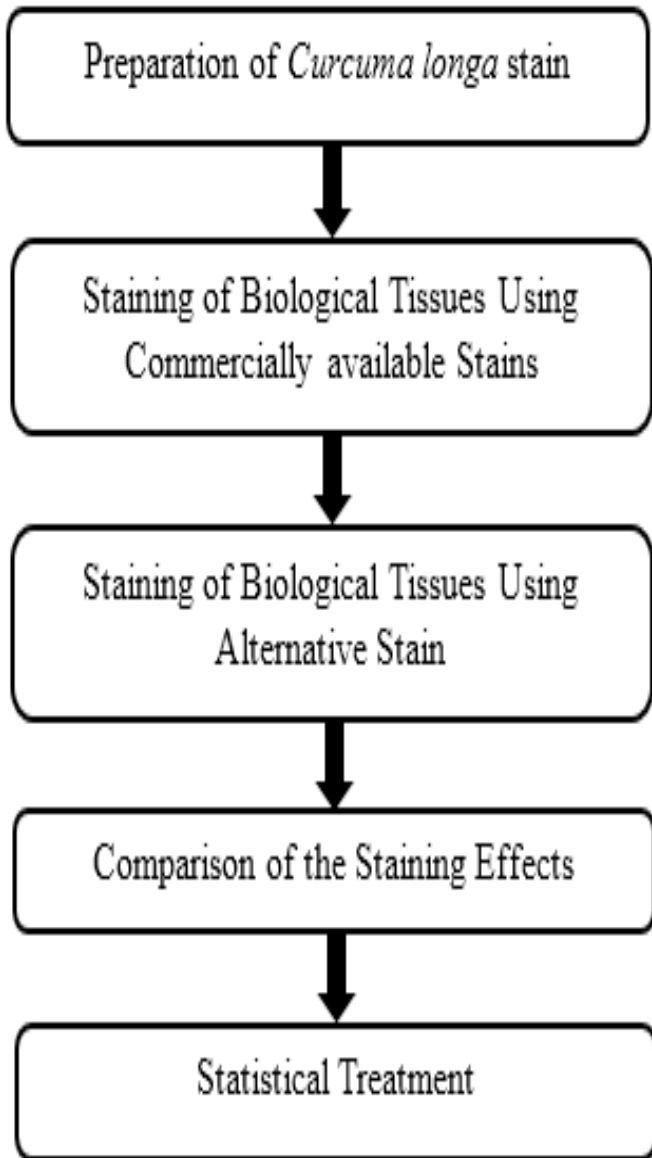
The conduct of this study aimed to examine the staining capacity of Turmeric extract on some plant and animal tissues. Specifically, this study answered the following questions:

1. What are the effects of the different concentrations of Turmeric in staining plant and animal tissues in terms of the following concentrations;
  - a) 15 grams in 100 mL of 70% alcohol,
  - b) 20 grams in 100 mL of 70% alcohol, and
  - c) 25 grams in 100 mL of 70% alcohol?
  
2. Is there a significant difference in the staining effects among the 15, 20, and 25-gram concentrations of Turmeric Stain on plant and animal tissues?
  
3. What is the staining effect of the of Turmeric extract on the following;
  - a) blood smear,
  - b) spinal cord,
  - c) cheek cell,
  - d) cycloid scale,
  - e) monocot stem,
  - f) dicot stem,
  - g) monocot root,
  - h) dicot root, and
  - i) leaf midrib?

4. Is there a significant difference in staining effect of the of Turmeric extract and the commercially available stain on the following;

- a) blood smear,
- b) spinal cord,
- c) cheek cell,
- d) cycloid scale,
- e) monocot stem,
- f) dicot stem,
- g) monocot root,
- h) dicot root, and
- i) leaf midrib?

**Materials and Methods**



**Fig 2:** Flowchart of the Study

**Preparation of *Curcuma longa* Stain.**

*Curcuma longa* L. rhizomes were collected from the local market in Malolos City, Bulacan, Philippines. It was cut into small pieces and sun-dried, then milled to form the fine powder. Fifteen, 20, and 25 grams of the powder was weighed using digital balance, which was dissolved in 100 mL of 70% alcohol in a beaker and was transferred to 5mL test tubes, which were centrifuged at 3000 rpm for 5 minutes and supernatant were collected in a coupling jar with the use of micropipettes.

**Staining of Biological Tissues Using Commercially Available Stains**

**Frog Blood Smear**

A drop of frog blood was placed on a slide and another slide was at one end at about 20° angle. It was then pulled o the right until it touched the blood. As soon as the blood had spread along the line of contact, it was pushed steadily by the right hand toward the left until all the blood disappeared or the other end of the slide was reached and dried the slides rapidly in the air by waving. Then enough Wright's stain was poured onto it, then was covered by the smeared portion of the slide. It was rinsed with tap water until the smear showed a pink tint in the thinnest part.

**Cheek Cell**

The mouth was rinsed with fresh water and disinfectant solution. A clean sterilized toothpick was obtained. It was used to gently scrape the inner side of the cheek to with the blunt end of the toothpick. The collected material was placed on a slide, and another slide of the same size was put upon it and pressed the upper cover gently with the eraser end of a pencil that made an even spread. Then the slides were quickly apart and air-dried. It was then stained with Gentian violet.

**Spinal Cord**

A fresh frog spinal cord cut into a small segment. It was placed on a slide then put another slide above the material. It was then pressed firmly but carefully upon the upper cover with the eraser end of a pencil. This squashed the soft tissue into a film. The sheet slid apart and air dried. It was stained with 1% aqueous solution of methylene blue for about two minutes.

**Cycloid Scale**

Scales of tilapia were gently scraped off and were preserved in alcohol. Small scales were selected and then stained with eosin.

**Plant Tissues**

Necessary plant parts (monocot and dicot root, monocot and dicot stem, and leaf midrib) were collected. A sharp double edge razor blade was obtained, and one edge of the razor blade was covered with masking tape. The knife was rinsed with warm tap water to remove traces of grease from the surface of the module. The plant material was held firmly. The article was pressed against the side of the first finger of the left hand (or right hand) employing the thumb. The first finger was kept as straight as possible, while the thumb was held well below the surface of the material out of the way of the razor edge. The razor was flooded with water that reduced the friction during cutting as sections floated onto the surface of the blade. The razor blade was take in the right hand (or left side) and placed on the first finger of the left hand (or right hand), more or less at a right angle to the specimen. Then, the razor was drawn across the top of the material in such a way as to give the article a drawing cut (about 45- in the horizontal direction). This resulted in less friction as the razor blade passed through the specimen. Several cross sections were cut at a time. The articles were transferred to water using a brush, not a forceps or needle. It was then stained with safranin for about 2 minutes.

**Staining of Biological Tissues Using Alternative Stain**

All biological tissues were prepared and stained using the

same procedure for both the commercially available and the alternative stains except for the dyes used.

**Comparison of the Staining Effect**

The staining effect of the different concentrations of turmeric stain was compared to the commercially available dyes. Three faculty members from the College of Allied Medical Sciences Department evaluated the stained tissues whether the staining effects are excellent, good, fair, or poor. The evaluators focused the attention to the critical structures in the different histological slides. For frog blood smear, the erythrocytes and its nucleus must be visible. For cheek cells, the cell membrane, nucleus, and cytoplasm should be observed while the chromatophore, focus, radii, and circuli of cycloid scale must be of sufficient appreciation. For spinal cord, the cell body of the multipolar neuron, cellular processes, and nucleus of glial cells should be seen. And in plant tissues, the vascular bundles (xylem and phloem), and dermal cells in the epidermis and endodermis have to be seen.

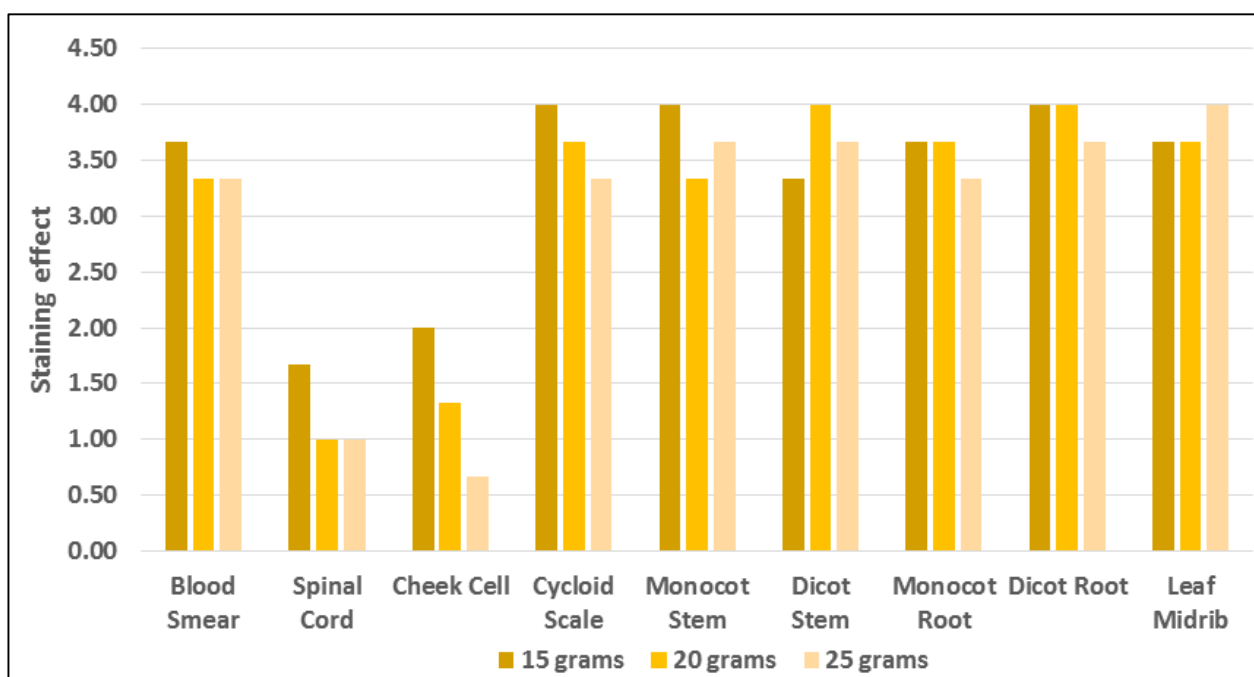
**Data Processing and Statistical Treatment**

The data collected in this study was subjected to one way analysis of variance which is commonly used to determine whether the differences can be attributed to one or a combination of independent variables. Specifically, one way ANOVA was used to assess the significant variations among the different independent groups which are the 15, 20, and 25 grams of turmeric stain and the chemically synthesized stain on various plant and animal tissues.

**Results and discussion**

**1) The staining effects of the different concentrations of Turmeric extract in plant and animal tissues in terms of the following:**

- a) 15 grams in 100 mL of 70% alcohol,
- b) 20 grams in 100 mL of 70% alcohol, and
- c) 25 grams in 100 mL of 70% alcohol.



**Fig 3:** The Mean Evaluation on Staining Effect of Commercial and Alternative Stains on Plant and Animal Tissues

Figure 3 shows the comparison of the staining effects of the different concentrations of *C. longa* extract to some animal and plant tissues. Most of the biological specimens stained using

15-gram concentration obtain the best results except for the dicot stem and leaf midrib.

Seven out of nine or 77.78% of the total biological specimens stained using the various concentrations of alternative stain yield a staining effect of 3.33, 3.67 and 4.0 which corresponds to an excellent staining effect except for the spinal cord and cheek cell

**2) The Staining Effect of Turmeric Extract in Different Concentrations.**

**Table 1:** ANOVA Results for Different Concentrations of *C. longa* Extract

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.564908	2	0.282454	0.2102	0.812106	3.4668
Within Groups	28.21849	21	1.343738			
Total	28.7834	23				

Table 1 shows the results of the analysis of variance on the staining capacity of 15, 20, and 25 grams of *C. longa* in 100mL of 70% alcohol solution. The *F*-value obtained in the calculation is less than the *F*-critical value. Therefore, it can be drawn out that the variations in the concentrations of alternative stain do not produce significantly different staining effects on plant and animal tissues. But among the different prepared concentrations, the 15 grams yield more excellent staining results.

In the study conducted by Kumar *et al.* (2014) [15], 5, 10, and 15 grams of pulverized turmeric was dissolved in 100ml of 70% alcohol and were used in staining epithelium, collagen, muscles, blood vessels, nerves, bone, adipose tissue, and keratin. Among those amounts of turmeric, the 15 grams provided the best results. Therefore, this quantity of turmeric may be set as the standard amount in preparing an alternative stain out of turmeric unless further related studies on the masses of turmeric will be conducted.

### 3) The Staining Capacity of Turmeric Extract on the Following Tissues

- a) blood smear,  
 b) spinal cord,  
 c) cheek cell,  
 d) cycloid scale,  
 e) monocot stem,  
 f) dicot stem,  
 g) monocot root,  
 h) dicot root, and  
 i) Leaf midrib.

**Table 2:** Staining Effects Means

Biological Specimen	Commercial Stain	15 g in 100 mL of 70% alcohol	20 g in 100 mL of 70% alcohol	25 g in 100 mL of 70% alcohol
Blood Smear	4.00	3.67	3.33	3.33
Spinal Cord	4.00	1.67	1.00	1.00
Cheek Cells	4.00	2.00	1.33	0.67
Cycloid Scale	4.00	4.00	3.67	3.33
Monocot Stem	4.00	4.00	3.33	3.67
Dicot Stem	4.00	3.33	4.00	3.67
Monocot Root	4.00	3.67	3.67	3.33
Dicot Root	3.67	4.00	4.00	3.67
Leaf Midrib	4.00	3.67	3.67	4.00

Legend:

0 - 1.0                      Poor      2.1 – 3.0    Good  
 1.1 – 2.0    Fair            3.1 – 4.0    Excellent

Table 2 shows the mean results of the staining effects of commercially available and turmeric stain on frog blood smear, spinal cord, cheek cells, cycloid scale, monocot and dicot root and stem, and leaf midrib. The staining effect means of chemically-synthesized stains on all plant and animal tissues obtained a numeric score of 4.0 except for dicot root which is 3.67. All numeric values obtained by staining effects of the commercially available stain corresponds to excellent staining results. However, the three varied concentrations of alternative stain had provided a staining effects of 3.33, 3.67, and 4.0 for frog blood smear, cycloid scale, and all plant tissues such as monocot and dicot root, and leaf midrib. The 3.33, 3.67, and 4.0 staining evaluation means produced by alternative stain is interpreted as excellent staining effect. Moreover, the staining effects of

alternative stain on spinal cord and cheek cells were 1.0 to 2.0 and would be interpreted as poor and fair.

### 4) Difference in the staining capacity of Turmeric extract and the commercially available stain on the following tissues

- a) blood smear,  
 b) spinal cord,  
 c) cheek cell,  
 d) cycloid scale,  
 e) monocot stem,  
 f) dicot stem,  
 g) monocot root,  
 h) dicot root, and  
 i) Leaf midrib.

**Table 3:** ANOVA Results for Comparison of Synthetic and Alternative Stains

Biological Specimen	F-value	P-value	F crit
Blood Smear	1.33	0.381282	6.59
Spinal Cord	33	0.002791	6.59
Cheek Cells	38.33	0.002095	6.59
Cycloid Scale	1	0.478949	6.59
Monocot Stem	3.67	0.120815	6.59
Dicot Stem	3.67	0.120815	6.59
Monocot Root	1.33	0.381282	6.59
Dicot Root	1	0.478949	6.59
Leaf Midrib	0.67	0.6151	6.59

It is shown in the Table 3 the computed *F-value* of the compared mean staining effects of commercially-available and alternative stains. Among all biological specimens, spinal cord and cheek cells got an *F-values* higher than *F-critical value* which are 33, and 38.33 respectively. Therefore, it suggests that the staining effects of commercially available stain is significantly different from the staining effects of alternative stain. For frog blood smear, cycloid scale, monocot and dicot stems and roots, and leaf midrib, the calculated *F-value* is lower than *F-critical value* and these correspond to no significant differences in the staining effect produced by commercially available and alternative stains. Therefore, the alternative stain may be used as substitute in staining those biological specimens.

### Conclusions

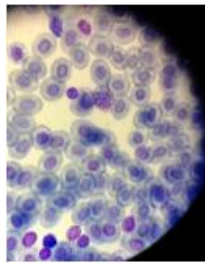
1. Different concentrations of turmeric stain can provide

2. excellent staining effects on frog blood, cycloid scale, monocot and dicot roots and stems, and leaf midrib.
2. There are no significant differences exist on the staining effects among the different concentrations of *C. longa* in 100 mL of 70% alcohol.
3. Alternative stain may be used in lieu of commercial stain in staining frog blood, cycloid scale, monocot and dicot roots and stems, and leaf midrib.
4. There are no significant differences exist on the staining effects of *C. longa* stain and commercial stain on plant and animal tissues except for cheek cells and spinal cord.

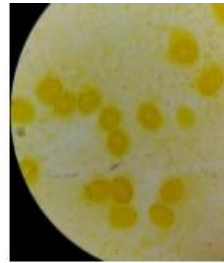
### Acknowledgement

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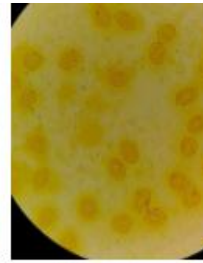
Wright Stain



15-gram  
Concentration

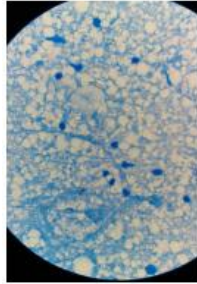


20-gram  
Concentration



25-gram  
Concentration

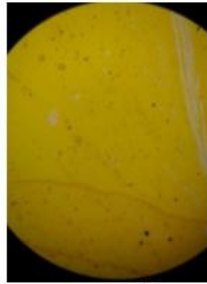
Frog Blood Smear



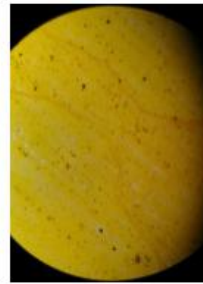
Methylene Blue



15-gram  
Concentration



20-gram  
Concentration

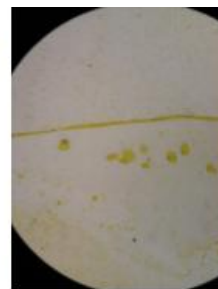


25-gram  
Concentration

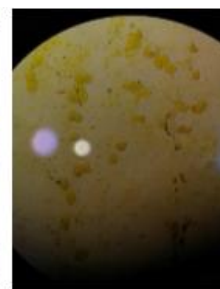
Frog Spinal Cord



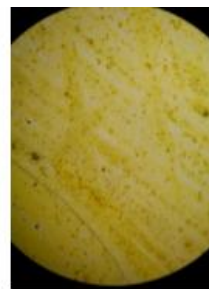
Methylene Blue



15-gram  
Concentration

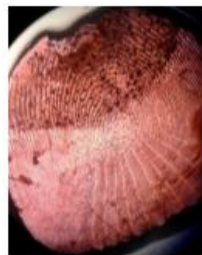


20-gram  
Concentration



25-gram  
Concentration

Cheek Cells



Eosin



15-gram  
Concentration



20-gram  
Concentration

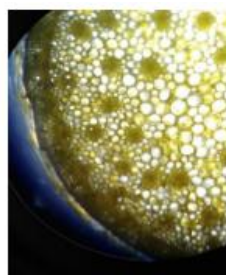


25-gram  
Concentration

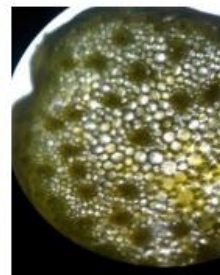
Cycloid Scale



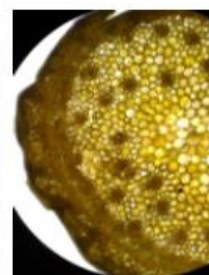
Safranin



15-gram  
Concentration

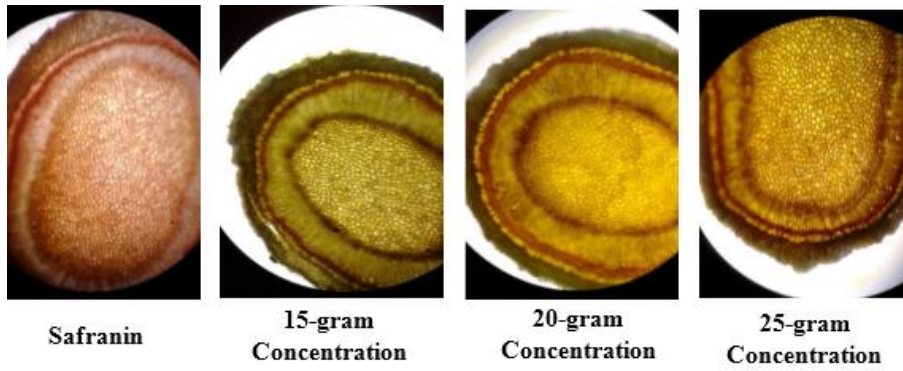


20-gram  
Concentration



25-gram  
Concentration

Monocot Stem



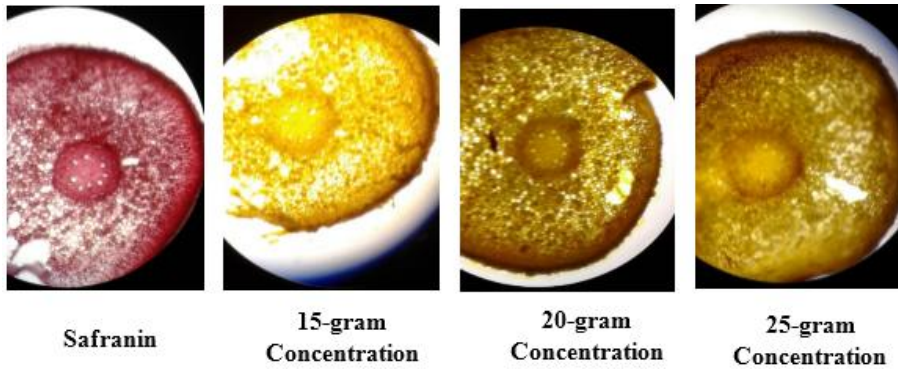
Safranin

15-gram  
Concentration

20-gram  
Concentration

25-gram  
Concentration

Dicot Stem



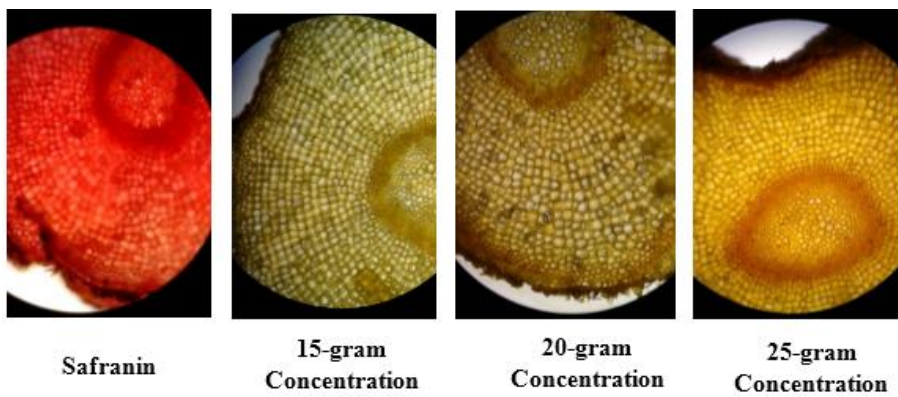
Safranin

15-gram  
Concentration

20-gram  
Concentration

25-gram  
Concentration

Monocot Root



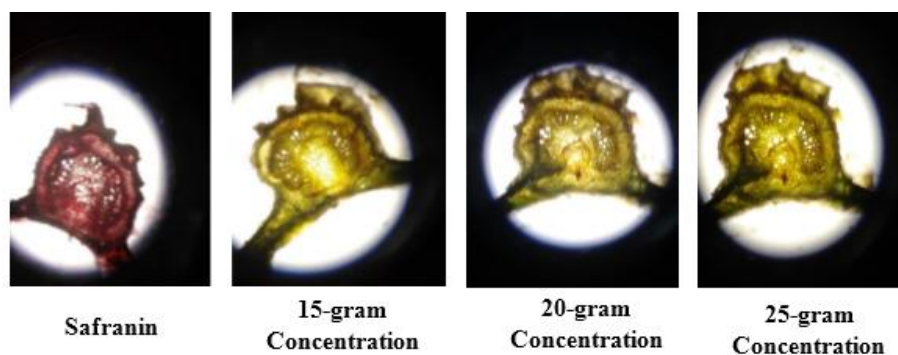
Safranin

15-gram  
Concentration

20-gram  
Concentration

25-gram  
Concentration

Dicot Root



Safranin

15-gram  
Concentration

20-gram  
Concentration

25-gram  
Concentration

Leaf Midrib

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