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Research Article

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Dyeıng Effect of *Brassica Oleracea* Extract in Onıon Roots and Human Blood Cells

Nilgün Güler Kuşçulu[1]*, Güzin H. Arslan[2]

[1]Department of Chemistry Technology, Mustafa Çıkrıkçıoğlu Vocational School, Erciyes University, Kayseri, Turkey
[2]Department of Chemistry, Faculty of Science, Erciyes University, Kayseri, TURKEY

[*]**Corresponding author:** Nilgün Güler Kuşçulu, Department of Chemistry Technology, Mustafa Çıkrıkçıoğlu Vocational School, Erciyes University, Kayseri, Turkey. Tel: +904380243803; Email: nguler@erciyes.edu.tr

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Abstract

Environmentally friendly natural dyes have been used in food, textile, medicine fields. The red cabbage (Brassica oleracea) plant including cyanidin is usually used as food material. However, due to the presence of anthocyanins, cyanidin can be evaluated as a source of dyes. The cells were examined by staining. Onion roots and human blood used to disseminate the use of plant-derived dyes and to show the staining of the nuclei of different cells. The alum also used as a mordant material for bonding between cell core and dyestuff. Furthermore, only onion roots treated with salicylaldehyde for been having cell walls and then taken to the dye solution. A few drops of blood taken from healthy, voluntary 20-years old person spread peripherally. A top of onion provided from a local market immersed in a small beaker of water and its roots put into 70% ethyl alcohol. 20% red cabbage extract prepared. 1 piece of onion root and blood preparation put into the extract. They subjected firstly at room temperature and secondly 50° C for one-hour to dyeing processes. In addtion, one of the onion root immersed in the dye solution after standing in salicylaldehyde. The other root directly added to the dye solution. Both solutions kept at 50° C for 1 hour. After staining, onion root cells and blood cells washed. They examined as × 40, × 100 magnitudes in the light microscope. No staining observed in both cell types at room temperature. When the temperature of the dye bath increased, a blue coloring observed in the nucleus of onion stem cells. Especially erythrocytes in blood cells stained dark blue with alum. No staining observed in the onion stem cells treated with salicylaldehyde. That is, the onion roots stained in the dye bath containing alum mordant without salicylaldehyde.

Keywords: *Cell Staining; Cyanidin; Dyestuff; Mordant; Red cabbage*

Introduction

There are four primary sources from which natural dyes are available: plants, animals,minerals and soil [1,2]. Many plants and some animals have been identified as potentially rich in natural dyes or as dye producers and some of them have been used for natural dyeing for many years [2,3].Natural dyes are mostly derived from plant sources and they have been used in the textile, food, cosmetic, pharmaceutical and histological fields [4,5]. When there was no direct interaction between cell and the dyestuff, various metal salts which are known as mordant agents are used like $KAl(SO_4)_2\,12H_2O$, $FeSO_4$, and $CuSO_4$, so that bright and dark colored stainings are obtained in cell [6,7].

Some natural biological dyes such as carmine from cochineal (*Dactylopius coccus*) and hematoxylin from the logwood tree (*Haematoxylum campecianum* L.) have been used for microscopy [8, 9]. Another natural source is *Brassica oleraceae* whose leaves contain the cyanidine molecule which has antioxidant and vitamin properties and is shown in **(Figure 1)**.

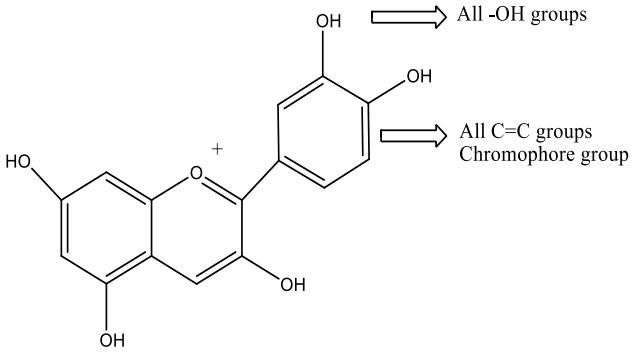


Figure 1: The Cyanidine structure (10).

Cyanidine has chromophore (colorants) and oxochromo (color enhancer and linker) groups. These groups are shown in **(Table 1)**. Therefore, it has good dyeing and an indicator property [10].

Chromophore Groups	Oxochromo Groups
Azo	Amino
Carbonyl	Substitue Amino
Nitro	Hydroxyl
	Thyoalcohol
Ethylene	Metoxy
Thiocarbonyl	Sulphonic
Nitroso	Phenolic

Table 1: Structure of oxo and chromophore groups.

There are various proteins in the eukaryotic cell structure. But histone proteins are only found in the cell nucleus. Therefore, histone proteins have been thought suitable for coloring according to dyeing results which occure only in the nucleus region [11,12].

In our previous study, we obtained dye from madder root extract and we stained chromatin in the onion root cell nucleus and highest temperature [13]. In this study, the dyeing of chromatin occurred in the nucleus of onion root cell by using different plant extract and the lowest temperature. But dyeing was not seen in nucleus of the blood cells. Because, only erythrocyte which has no nucleus from the blood cells were dyed In this study, the dyeing process occurred in the nucleus by using different plant extracts and the lowest temperature. At the first step staining of onion root cell with *Brassica oleraceae* extract conducted with alum and without alum. At the second step, onion root assembled with a number of chemical processes and then this cell stained in *Brassica oleraceae* extract with / without alum. Blood cells also stained without alum at 50°C for one hour. Therefore, the objective of this study was to investigate the extraction of natural dye from *Brassica oleraceae* with distilled water and using alum, salicylaldehyde, and then to examine its staining property on the nucleus of onion root cell and blood cells at suitable pH and temperature.

Materials and Methods

Materials and instruments

All the common laboratory chemicals used in the synthesis of the substance purchased from commercial sources and used without further purification. $KAl(SO_4)_2$ $12H_2O$ (alum) is Merck Millipore. Ethyl Alcohol is pure and Sigma Aldrich.Onions supplied from the local markets.

Preparation of plant extract

A twenty-gram of Brassica oleraceae, which obtained from Bafra (Samsun, Turkey) homogenized using an IKA T-18 homogenizer. It placed in a beaker and 100 milliliters (w/v 20 %) of distilled water added. The mixture boiled on a hot plate for 30 minutes and filtered. The filtrate stored in the refrigerator at 4°C and used for staining.

Preparation of onion root

The top part of the onions dipped in a beaker with water. After two days, when the new roots appeared, approximately 1- 1.5 cm of the root tips cut off and transferred to a glass bottle containing with 70 % ethyl alcohol (Sigma Aldrich). The root tips stored at 4°C until dyeing.

Preparation of a peripheral blood film

One drop of blood placed on the surface of the first labeled slide, near the frosted end. Placed the second labeled slide in contact with the first, so that the drop of blood was inside an acute angle between the two slides. If the drop of blood scant, adjust the slide so that the angle between the two was as fast as possible. If the drop was particularly copious, increased the angle.Then, the slides dried in the air. Fixative solution (70 % ethyl alcohol) dripped on to slides and they held at room temperature to dry. Figure 3a is included ımage of the slide taken by light microscopy.

Dyeing of onion root cells

Three eppendorf tubes used. The double experimental set up was as follows. One mL of dye extract added to the first tube, 1 mL of dye extract and 0.05 g alum added to the second tube, and 2 pieces of onion root placed into each tube. Each tube placed in water baths, at 50°C. Incubation was for 90 minutes duration. At the end of dyeing incubation time, the root tips washed with distilled water then placed onto clean microscope slides. After putting cover slides onto the microscope slide, the onion root piece squashed between the slide and the cover slide. Photographs of the cells on the slides used for observation and taken by light microscopy. These photographs are shown in **(Figure 2)**.

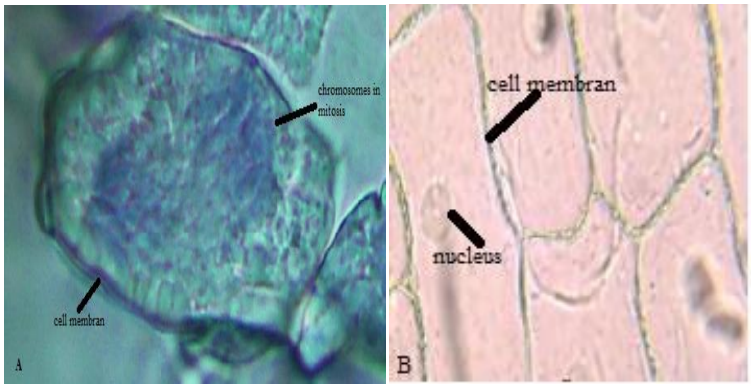


Figure 2: Images of onion root cells dyed with Brassica oleraceae extract ×100. (A) With alum (B) Without alum.

Reaction of onion root cell with salicylaldehyde

Each onion root piece subjected to a reaction with salicylaldehyde in 10 % ethyl alcohol at above 50°C using an magnetic stirrer for seven days. The progress of the reaction which measured by the presence of the salicylaldehyde's odor and the structure of the onion root pieces followed by observation with a microscope. At the end of the seventh day it observed that no reaction had occurred. Therefore, the reaction repeated by crushing onion root pieces in an agate mortar. The reaction terminated when the characteristic odor of aldehyde disappeared and the more swollen state of the onion roots observed. The cell dyeing procedure applied to onion root pieces which treated in this way.

Dyeing of blood cells

A 100 mL extract of the plant distributed to the beakers in portions of 50 milliliters. One of the beakers left empty, the others loaded with 2.0 grams of alum mordant ($KAlSO_4.12H_2O$) and mixed with glass baguette. Each slide submerged to each beaker. The dye solution put into oven at 50°C for 1 hour. 1 hour later, the slides removed from the dye solutions and washed with distilled water, and left to air-dry. Photographs of the blood cells on the slides taken at 10 × 40 magnification by light microscopy.

Results

The cell nuclei obtained from onion roots were stained with *Brassica oleraceae* extract in alum media. We understood that the staining without alum media at room temperature would not succeed. This was evident from the microscopic examination. Figure 2 (a and b) also shows stained with *Brassica oleraceae* extract the pictures of the cells nucleus derived from onion root in alum and non alum media. As seen from the pictures the nuclei of the onion root cells stained with *Brassica oleraceae* extract are more clear and the cell walls are smoother.

The dyeing temperature determined as 50°C so as not to cause the denaturation of the cell structure. Staining did not observe at temperatures below 50°C. We did not conduct studies at 80°C because of the denaturation of cells. *Brassica oleraceae* extract has a dyestuff feature, because of the –OH groups of the cyanidine molecule. These interact with the metal atoms in metal salt so chelate complexes occur and the resulting chelate complexes are colored in the visible region. Therefore the nuclei of the onion root cells stained with *Brassica oleraceae* extract and alum observed to be a blue color in light microcopy. As a result of the reaction with salicylaldehyde of the onion root cells dyeings realized in alum or non alum media. In this case salicylaldehyde used in the reaction with NH_2 which belongs to the R group of aminoacids in the cell nucleus and shutted to the effective

region, It could not interact with the cells in this region, so chelation did not occur. For this reason, dyeing did not realize.

When the NH_2groups of onion root cells reacted by salicylaldehyde, they were inactive. Inactivated cells stained with *Brassica oleraceae* extract. Staining of the cell nucleus in alum and non alum media did not observe. At this position, the NH_2groups of the histone protein's aminoacids which are numerous in the cell nucleus are reacted with salicylaldehyde then, cessation of the chemical interaction with dyestuff is indicated. Salicylaldehyte prevented staining. Cytogenetic studies are being carried out on the mutational effects of some chemicals, insecticides on plants [14]. So that, *Brassica oleraceae* extract with alum can be use as dye source in cytogenetic studies on root tip of *Allium cepa* L. Staining results of blood cells are shown in **(Figure 3)**. In first image, blood cells did not stain to compare with dyed images. In other images alum mordant put and not put into the *Brassica oleraceae* extract to see effect of alum.

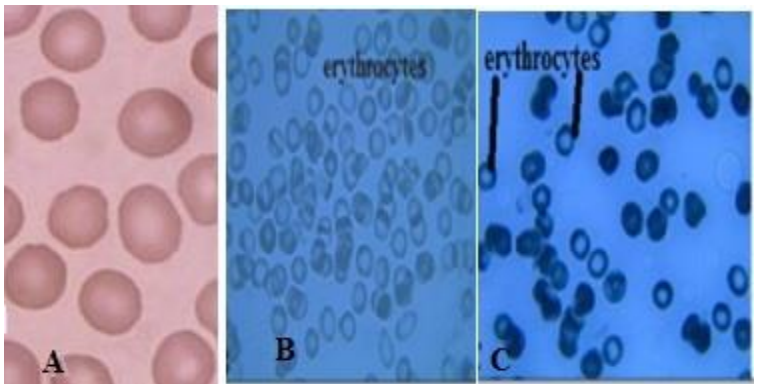


Figure 3: Images of blood cells ×100 (A) Without Brassica oleraceae extract (B) Without alum in Brassica oleraceae extract (C) With alum in Brassica oleraceae extract.

Discussion

In this study, onion root cells stained with *Brassica oleraceae* extract. The optimum conditions determined as 50°C and a pH range of 6-8. At the same time, this pH range is suitable for studying a range of many complex formation reactions. At the first stage, nucleus of the onion cells which stained with *Brassica oleraceae* extract is seen most blue. when we only used alum mordant in *Brassica oleraceae* extract. In the second step, cells reacted with salicylaldehyde. A schiff base used in this study because the salicylaldehyde, which is a base, is harmless to the cell nucleus and did not detect as a mutagenic substance by the study conducted [15]. Besides, it is known that schiff bases dye wool by complexing with Cu metal, dyeing with *Brassica oleraceae* extract did not occur in the cells to which we applied salicylaldehyde before dyeing [15,16]. Because the schiff bases make themselves complex, they take up the stain. In this case, it is not appropriate to use schiff bases as an enhancing agent for dyeing.

As a result of this reaction, after physical processes, some physical changes in the onion root cells observed not to ocur when dyeing in alum and non alum media. This resulted from the amino acid ends of the histone proteins which reacted with salicylaldehyde and inactivated. *Brassica oleraceae* has some advantages as a dye source. It is easily found in every city or country, cheap and has more color density. When we saw staining in onion cell nucleus and blood cells. Cell nucleus of onion was dyed by *Brassica oleraceae* at low temperature for 1 hour. So that, we thought to have been dyeing of alkaline histone proteins by asidic dye molecules in *Brassica oleraceae* extract with alum mordant at 50 ° C for 1 hour. Because DNA molecule has asidic property.

Dyeing is a process which depends on the interaction between the dye and the material to be dyed. These interactions are electrostatic interactions and complex formation between acid and base molecules. In this study, two factors play a role obtaining the positive result at low temperature of dyeing. The primary factor is the acid-base attraction between the dyestuff and the nucleus of the cell the chelat formation from the added salt between the dyestuff and histone proteins to facilitate colouration. The reason for not seeing the same effect in the blood cells is that the density of erythrocytes in the cell nucleus is high and their nuclei are not. The dyestuff molecules and the mordant are thought to be in less contact with the nucleated cells in the cell.

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