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Staining of Onion and Buccal Epithelial Cells with Onion Skin Extract

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Research Article

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ABSTRACT

The aim of our study is to reveal the stainability of different parts of buccal epithelial cells as an animal cell model, and onion membrane as a plant cell model with extract prepared from onion skin by means of light microscope images. After a 20 gram amount of the skins of clean and fresh headed onions with orange-yellow outer skins is ground, it is taken with precision weighing. This is added to 100 ml of purified water, boiled for 30 minutes, and filtered. The filtrate is stored in the refrigerator at +4°C for use as a dye solution. Solvents with separate binary combinations from the dye solutions alone and various metal salts (Alum, $FeSO_4$, $CuSO_4$) were prepared. Membrane pieces in the onion and buccal epithelial preparations were put into each solution. The staining process is finalized by keeping the prepared materials at 50°C and 80°C in an incubator for 1 hour, and then at room temperature for 1 hour. The onion membrane pieces and buccal epithelial preparations were then removed from the dye solutions, and rinsed out with pure water. Some membranes were placed on the slides and then covered with coverslips. Preparations including the painted buccal epithelial and onion membrane cells were examined under a microscope. It was observed that different sides of both the buccal epithelial and onion membrane cells were positively dyed in different colors and tones in the visible parts at different temperatures and in various mordants with the onion skin extract. Thus, it was determined that the aqueous extract of onion skin has potential in staining biological preparations under the conditions that gave positive results in this study.

INTRODUCTION

It has been known for many years that natural colorants have three sources such as plant, animal and mineral. The first known herbal staining source is madder (*Rubiatinctorum* L.), and the colorant is alizarin. In studies done later, various parts (leaf, stem, bark, flower, etc.) of many plants such as the rhamnus family, daisy family, venetian sumac, and gumwood were used as natural staining sources [1,2]. Animal staining sources are colorants obtained from American cochineal (*Dactylopiuscoccus Costa*), kermes (*Kermes vermilioPlanchon*) and lac (*Kerrialacca Kerr*) insects, which contain red colorants and have gained importance among many insect species. Chromophores, which are present in all insect-sourced colorants, have anthraquinonoid derivatives. Mineral stains are metal and metal salt mixtures called Prussian blue, chrome yellow, manganese bronze, and antimony oranges [3,4].

Efforts to utilize biomass around the world to provide healthier, more economical, easier and more affordable materials have long been known. In the light of the information that we obtained with our literature study, it was understood that the studies carried out in this field are not sufficient in spite of the size of the biomass. The edible parts and outer wastes of nutrient sources (such as onion: isorhamnetin and quercetin, broccoli: kaempferol and myricetin, apple: quercetin, mandarin: hesperetin) that are obtained from biomass contain many natural coloring materials [5,6]. As an agricultural country, Turkey has plenty of agricultural waste and product waste resources. Because these waste materials contain color matter, biomass, which is a very economical

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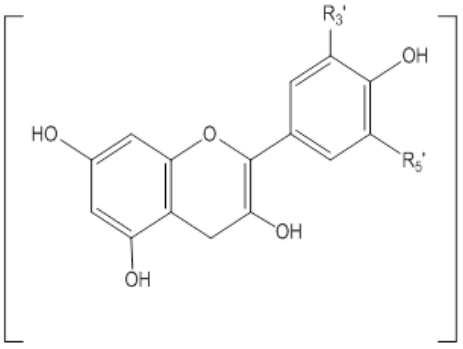
resource, is also harmless from the point of view of the environment and human health [7]. In addition to these properties, although many plants synthesize thousands of different kinds of coloring matter, very few of them have been studied for their potential as colorants. While some of these plants have been utilized as nutrients, it may be possible to investigate the coloring materials of certain parts, like skins and nucleus, by using appropriate isolation techniques, to develop new methods of staining with them, and to introduce more economical, and environmentally friendly histological dyes into the field of science and health [8].

The onion belongs to the genus Alum of the family Liliaceae. The dry skins of onion are used in staining. Pelargonidin (3, 5, 7, 4-tetra hydroxyanthocyanidol), a quercetin colorant is found in the outer skins of onions as well as pyrocatechin and benzocatechinic acids. As there are 4 hydroxy groups as well as oxychromes, which is a color enhancer group, such as NH_2 and COOH in the chemical structure of pelargonidin, it stains materials like wool very well and strongly [9]. It is one of the derivatives of anthocyanidin. The molecular structure of pelargonidin is as follows.

When there are chromophore groups in one molecule, the presence and number of OH and OCH_3 groups, known as oxochrome groups, increase color intensity. Accordingly, the color intensity of delphinidin, petunidin and malvinidin stains in **Figure 1** is higher than that of the others.



Anthocyanidins	R_3'	R_5'
Pelargonidin(Pg)	H	H
Cyanidin(Cy)	OH	H
Peonidin (Pn)	OCH_3	H
Delphinidin(Dp)	OH	OH
Petunidin (Pt)	OCH_3	OH
Malvinidin (Mv)	OCH_3	OCH_3

Anthocyanidins Pg, Cy, Pu, Dp, Pt, Mv

R_3: o -glucoside

R_5: o-glucoside

Figure 1. Chemical structure of pelargonidin in onion skin [10].

Some anthocyanin and natural phenolic compounds are used to make the cell core dye. Although, in particular, plants such as red cabbage, dahlia, blackberry juice, and black plum fruit, which contain anthocyanin, were identified for use as histological stains, it was preferred in this study, asin cytological and histological staining studies done before, to use onion skins as a plant colorant source. The reason why onion skins are preferred is that they are easy to find, renewable and cheap [11].

In this way, we aimed to determine the potential of onion skins for use as histological stains by activating the extract that we produced with water, which is the easiest molecule to pass through plant material and cell membrane, at room temperature, 50°C and 80°C, with onion and buccal epithelial cells, using the extract directly or with various metal salts.

MATERIALS AND METHODS

Preparation of Onion Skins

The skin of fresh, clean, yellow-orange colored onion bulbs which were supplied from Talas region markets in Kayseri and were collected in a separate cloth bag. It should be noted that the skins were collected from clean, dry and dark colored parts.

Preparation of Plant Extract

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

Preparation of Buccal Epithelial Cells

Two drops of distilled water were dropped onto a slide, and the epithelium cell, which was taken from a healthy 20 year young woman, was removed by digging it out with two separate toothpicks from the inner part without causing damage. The toothpicks were turned in the water drops on the slide to allow the cells to pass into the water. The water on the slide was removed by gently heating it in a burning flame, and the cells were fixed with fixative (methanol: acetic acid) (3:1).

Staining of Onion and Buccal Epithelial Cells

A 100 mL plant extract was distributed to beakers in portions of 25 milliliters. One of the beakers was left empty; the others were loaded with 0.5 grams of three different mordant materials ($KAlSO_4.12H_2O$, $FeSO_4$, $CuSO_4$), and mixed with a glass rod. In each one, one piece of onion skin and a piece of slide containing buccal cell were dipped. The pH value of each solution was measured and noted. The paint solution was allowed to stand at room temperature for 1 hour, while the other dye solutions prepared in the same way were kept at 50°C and 80°C for 1 hour. One hour later, the slides were removed from the dye solutions

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and washed with distilled water, and left to dry. The Onion bulbs were washed, taken onto a clean slide, and covered properly with lamella. The onion and buccal epithelial cells were subjected to experimental sets with different parameters as shown in **Table 1**, and the slides were then evaluated by light microscope.

Table 1. Cell staining conditions with different temperatures and mordants with onion skin extract.

Plant Extract Name	Cell Type (Membrane+ epithelium)	Temperature	Duration	pH	Mordant Name(0.5 g)
Onion skin	Onion + buccal epithelium	25°C	1 hr	4	-
Onion skin	Onion + buccal epithelium	50°C	1 hr	4	-
Onion skin	Onion + buccal epithelium	80°C	1 hr	4	-
Onion skin	Onion + buccal epithelium	25°C	1 hr	2	Alum
Onion skin	Onion + buccal epithelium	50°C	1 hr	2	Alum
Onion skin	Onion + buccal epithelium	80°C	1 hr	2	Alum
Onion skin	Onion + buccal epithelium	25°C	1 hr	3	$FeSO_4$
Onion skin	Onion + buccal epithelium	50°C	1 hr	3	$FeSO_4$
Onion skin	Onion + buccal epithelium	80°C	1 hr	3	$FeSO_4$
Onion skin	Onion + buccal epithelium	25 °C	1 hr	3	$CuSO_4$
Onion skin	Onion + buccal epithelium	50 °C	1 hr	3	$CuSO_4$
Onion skin	Onion + buccal epithelium	80°C	1 hr	3	$CuSO_4$

RESULTS

Staining Results of Onion Cells with Onion Skin Extract

In **Figure 2**, there is orange staining on the nucleus and yellow staining on the membrane without alum, dark yellow staining on the nucleus and light yellow staining on the membrane with alum, green-brown staining on the nucleus and the membrane with $FeSO_4$, reddish-brown staining on the nucleus and yellow staining on the cytoplasm and membrane with $CuSO_4$. All staining results are positive with onion skin extract on onion membrane cells at room temperature.

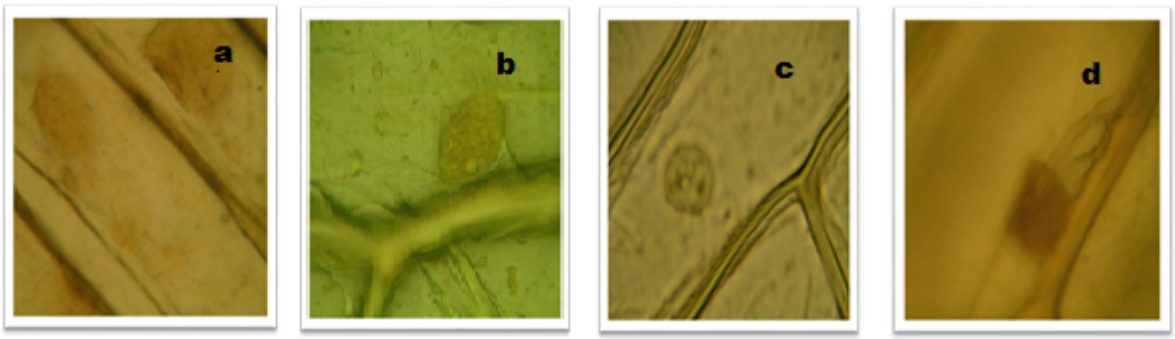

Figure 2. At room temperature, onion membrane cells dyed in onion skin extract (a) without alum, (b) with alum, (b) with $FeSO_4$, (d) with $CuSO_4$ (light microscope images 100X).

In **Figure 3**, there are brown-stained parts on an orange background on the nucleus and orange staining on the membrane without alum, green-stained molecules on a yellow staining background on the nucleus and light yellow staining on the membrane with alum, regional dark green staining on brown background on the nucleus and light green on the membrane with $FeSO_4$, While some parts were colored cinnamon on an orange background on the nucleus. There was orange staining on the membrane of onion cell with $CuSO_4$ in onion skin extract at 50°C.

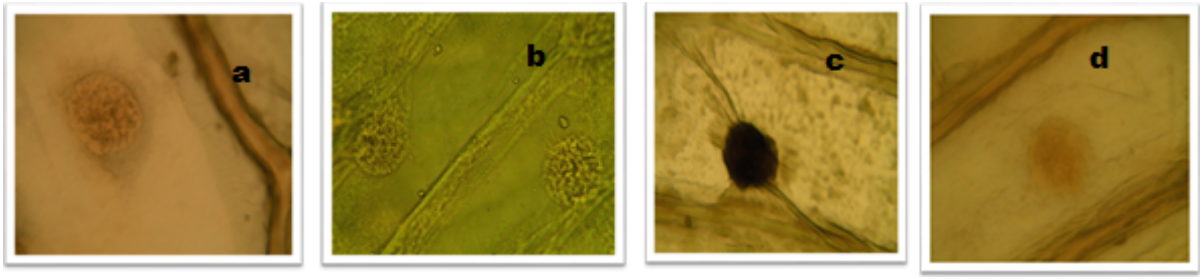

Figure 3. At 50°C, Onion membrane cells dyed with onion skin extract (a) without alum, (b) with alum, (c) with $FeSO_4$, (d) with $CuSO_4$ (light microscope images 100X).

In **Figure 4**, it was observed on the onion skin cells with onion skin extract at 80°C that there was yellow staining on the cell membrane without alum, orange staining on the nucleus and membrane with alum, dark brown-yellow staining on the nucleus

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and light yellow on the membrane with $FeSO_4$, and dark orange staining on the nucleus and membrane with $CuSO_4$. Positive staining results were obtained with all the metal salts on the nucleus and membranes of the onion cells at this temperature.

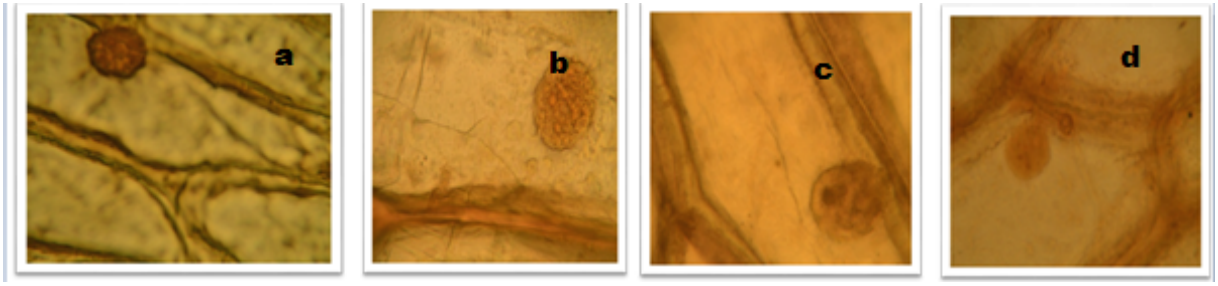

Figure 4. At 80°C, Onion membrane cells dyed in onion skin extract (a) without alum, (b) with alum, (c) with $FeSO_4$, (d) with $CuSO_4$ (light microscope images 100X).

Staining Results of Onion Epithelial Cells with Onion Skin Extract

In **Figure 5a**, there are bright yellow stained areas on the nucleus and cytoplasm. In **Figures 5b-5d**, yellow staining was observed on the nucleus and cytoplasm while darker brown staining on the nucleus and light brown staining on the cell cytoplasm were observed.

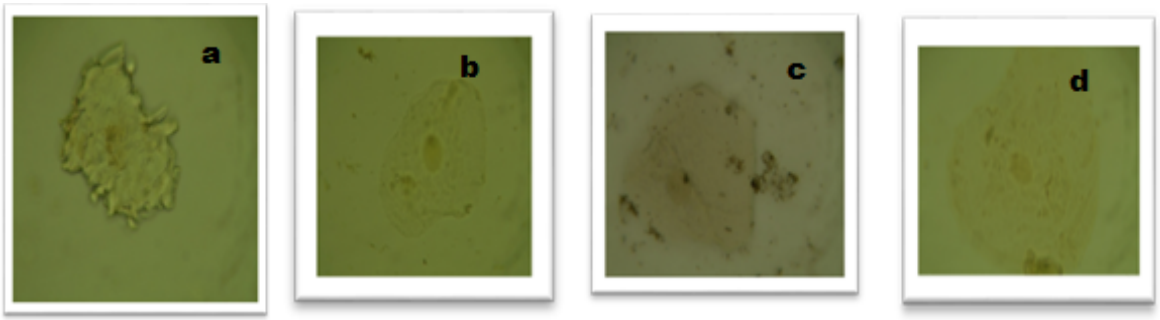

Figure 5. At room temperature, buccal epithelial cells dyed in onion skin extract (a) without alum, (b) with alum, (c) with $FeSO_4$, (d) with $CuSO_4$ (light microscope images 100X).

In **Figure 6a**, there is yellow staining on the cytoplasm. In **Figure 6b**, dark yellow staining was observed on the nucleus more intensely whereas dark brown staining on the nucleus and light brown staining on the cytoplasm were observed in **Figure 6c**, and orange staining was observed on the nucleus and cytoplasm in **Figure 6d**.

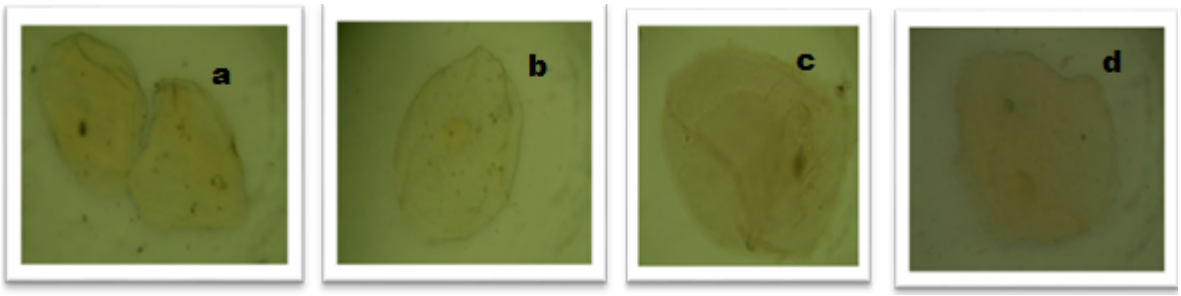

Figure 6. At 50°C, buccal epithelial cells dyed in onion skin extract (a) without alum, (b) with alum, (c) with $FeSO_4$, (d) with $CuSO_4$ (light microscope images 100X).

In **Figure 7b**, which shows the result of staining of the buccal epithelial cells with onion skin extract, there are yellow stained regions in the nucleus and cytoplasm whereas light yellow stained regions are observed in the cytoplasm in **Figure 7a**. In **Figures 7c** and **7d** dark green and orange-brown staining's are observed in the cell nucleus and cytoplasm, respectively.

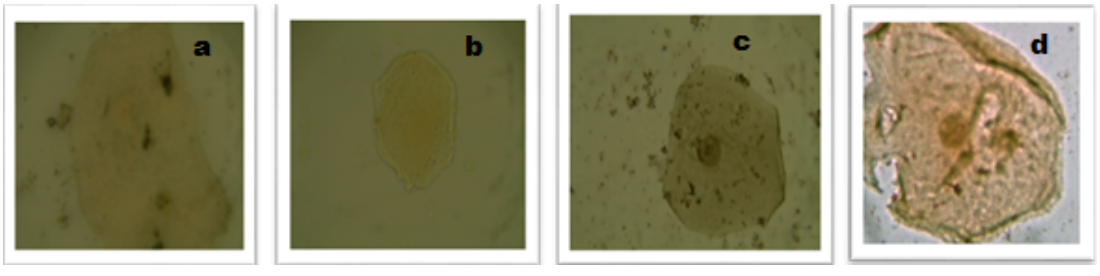

Figure 7. At 80°C, buccal epithelial cells dyed in onion skin extract (a) without alum, (b) with alum, (c) with $FeSO_4$, (d) with $CuSO_4$ (light microscope images 100X).

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As can be seen in **Table 2**, successful orange-yellow color stainings were observed on the cell nucleus and cytoplasm of the onion cells, which are plant cells, with staining extract alone obtained from onion skins after staining at room temperature, 50 and 80 degree temperatures whereas only yellow staining was observed on the nucleus and cytoplasm of the buccal epithelial cells at room temperature. Even when the temperature is increased, no staining was observed in the nucleus of the buccal epithelial cells.

Table 2. Staining results in onion and buccal epithelial cells at different temperatures with onion skin extract.

	At Room Temperature		At 50°C		At 80°C	
Cell Type	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm
Buccal Epithelial	Yellow	Yellow	Colorless	Yellow	Colorless	Yellow
Onion Membrane	Orange	Yellow	Orange	Orange	Orange	Yellow

In staining the buccal epithelial cells at room temperature, as shown in **Table 3**, the use of different mordants caused the cell nucleus and membrane color to turn from light yellow to darker yellow and orange, even brown. Positive staining results were obtained in the nucleus and membrane of the cell in staining trials of onion cells with and without mordants.

Table 3. Results of staining on onion and buccal epithelial cells using different metal salts with onion skin extract.

	Alone Extract		Extract with Alum		Extract with $CuSO_4$		Extract with $FeSO_4$	
Cell Type	Nucleus	Cell Membrane	Nucleus	Cell Membrane	Nucleus	Cell Membrane	Nucleus	Cell Membrane
Onion Membrane	Orange	Yellow	Dark Yellow	Yellow	Orange	Orange	Brown	Brown
Buccal Epithelial	Light Yellow	Light Yellow	Dark Yellow	Dark Yellow	Orange	Orange	Brown	Brown

We understood that the stainings of onion cells with mordants or without mordant media and stainings at 50°C and 100°C would be completely successful. However, stainings of buccal epithelial cells would only be successful with some mordants, at some temperatures. As a result, onion skin has potential for staining some biological materials. It is an ecological and very cheap dye source.

DISCUSSION AND CONCLUSION

Due to the fact that the colorants in the structure of plants have different organic structures, the types of solvents in which they dissolve also vary. For example, pelargonidin and quercetin colorants on onion skins have strong solubility in water. As the onion skins give intense color molecules in water, positive results were obtained in the staining of onion and buccal epithelial cells which we made with aqueous extracts. In addition, as phenolic, not polyphenol, pelargonidin and flavonol group quercetin colorants are present in the onion skin, their staining colors and intensity are different. Therefore, the onion skin dye could stain the nucleus and cytoplasm of onion and buccal epithelial cells with mostly orange-yellow colors.

The optimal staining condition of onion cells was found to be from the skins of onion dye extracted (1:5 skin: solvent, w/v) by distilled water for 60 minutes at room temperature, 50 and 80°C, with or without mordant. For buccal epithelial cells, the optimal cytoplasm staining condition was found to be from onion skin dye extracted by distilled water for 60 minutes at 50 and 80°C without mordant. At the same time, the optimal nucleus staining of buccal epithelial cells was obtained with only $CuSO_4$ and $FeSO_4$ mordants, at room temperature, 50 and 80°C. In these conditions, onion skins could be used as an alternative natural dye for histological stainings. Hence, for further studies, it is highly recommended that the conditions and methods required for the dyeing of different cells with various natural dye sources should be investigated.

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