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# Utilizing synthetic juices as makeshift histological stains – A novel approach

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## Abstract:

Context: At times such as the present COVID-19 Pandemic, wherein much of the economic resources are diverted towards acquiring protective personal equipment for the healthcare personnel, it becomes difficult to procure and maintain laboratory supplies. Thus, there arises a need to develop alternatives which are easily available and economically feasible for various chemicals used in histotechnology laboratories such as stains as well.

**Materials and Methods:** Synthetic juices of Orange, Rose and Kala Khatta flavors respectively were obtained from the local market. 4 µm thin sections were obtained from Formalin-fixed Paraffin-embedded tissues of departmental archives. The sections were deparaffinized, rehydrated and immersed in respective Coplin jars filled with staining solutions for five minutes. The stained sections were then washed under water, dehydrated, cleared, mounted with DPX and observed under light microscope.

**Results:** All the solutions caused non-selective staining of the histological section, however, in varying shades of respective colors (orangish, reddish pink and purplish) such that various tissue components could be easily differentiated. Characteristic stainings resulting from the solutions included bright orange staining of keratin by Orange solution and prominent dark purple staining of fibrovascular elements by Kala Khatta solution.

**Conclusion:** Synthethic juices could be effectively and efficiently used as eco-friendly and economically feasible makeshift histological stains for a rapid screening and diagnosis in histopathology. Further research is warranted with regards to standardization of these solutions to be utilized for histologic staining and their staining characteristics in various pathological tissues.

## Introduction:

The term 'Staining' implies utilization of a marker of characteristic color or form for visual labelling of a biological entity by attaching to it or depositing in its vicinity [1]. The so-called 'marker' or the reagent used to generate it is listed as a 'Stain' in nomenclature. Ever since the advent of hematoxylin and eosin, the stains have invariably been used as gold standards for histological staining of sections for diagnostic purposes [2]. Undoubtedly, these standard stains are diagnostically superior, however, they are not free of certain limitations [3].

Although most commonly employed for staining, the tediousness in preparatory procedures and procurement of these routinely used stains must not be underestimated.

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Another challenging aspect in utilization of these stains is their relative technique sensitivity and variation in staining characteristics on modification of even the slightest aspects of staining procedure. Additionally, these stains are usually obtained by limited number of specialized seller and are also relatively high-priced [4].

The difficulty of procurement of such stains becomes even more amplified at times such as the present COVID-19 pandemic. There is already an existing difficulty in maintaining the laboratory supplies at such times and the focus of much of the economic resources is shifted towards provision of essential protective equipment to the laboratory personnel [5]. Emphasis has been laid in laboratories worldwide to develop and utilize easily available, non-toxic chemicals that are friendly to the environment, with a similar need arising in histological staining procedures [6].

In this context, we attempted to utilize certain synthetic juices that contain food coloring dyes for histological staining so as to observe their staining characteristics. The study was conducted with an objective to open up possibilities in developing makeshift histological stains which are economically feasible and easily available, especially in situations where procuring standard stains becomes difficult or unfeasible.

## Materials and methods:

*Selection and procurement of synthetic juices:*

Synthetic juices of Orange, Rose and Kala Khatta flavor containing orange, pinkish and purplish food coloring dyes respectively were obtained from the local vendors in the city of Mumbai for the purpose of this study. Care was taken to ensure that the juices were not contaminated or diluted with additional ice. The juices were stored in a leak-proof clean container under refrigeration at 4°C until transporting them to the histopathology laboratory.

Preparation of histologic sections:

Formalin-fixed Paraffin-embedded blocks of oral tissues obtained from patients that had undergone crown lengthening procedure were selected for the purpose of this study and procured from the archives of the Institutional Department. The particular selection was done to ensure that none of the

valuable pathological tissue was misspent as the histopathologic staining potential of the juices was yet unconfirmed. Sections of 4µm thickness were taken from the selected specimen blocks by means of a semi-automated microtome (Leica Biosystems, Germany).

Staining Procedure:

The sections were deparaffinized and rehydrated to water. At this point, the juices were transferred to a Coplin jar wherein they would be hereafter referred to as 'staining solution'. pH of the juice was measured by means of a digital pH meter immediately following which the slides were introduced into the staining solution. The timing and pH were promptly noted down at the time of beginning the procedure. The slides were removed from the Coplin jar after 5 minutes of staining. Post-staining:

After removing the slides from the staining solution, they were briefly rinsed with water for 2-3 seconds in order to remove excess residual droplets of stain evident on the slides. Care was taken not to let excessive amount of water come into contact with the slide that would cause reduction in staining intensity owing to the water-soluble nature of food coloring dyes. The sections were then cleared with xylene, mounted with DPX and observed under light microscope.

## Results and Discussion:

*A) Staining characteristics with synthetic orange juice:*

The sections were stained non-selectively in varying shades of orange color (Figure 1). However, the various components of oral epithelium and underlying connective tissue could still be easily differentiated without any confusion. This could be because of the selective uptake of the dye molecules by different components of tissue having differing permeabilities and attachment sites. The basement membrane stained prominently dark orange clearly defining the boundary between epithelium and connective tissue stroma. The cytoplasm of the epithelial cells had a lower intensity of staining as compared to the basement membrane with a very faintly staining cell membrane, which caused clear demarcation of the epithelial cells. The upper layers of the stratified squamous epithelium i.e. the stratum corneum and keratin stained as bright orange with even lower intensity. Collagen fibers and blood vessels also stained with a darker shade of orange which could be clearly demarcated from the surrounding extracellular matrix.

*B) Staining characteristics with synthetic rose syrup:*

Non-selective pinkish to reddish staining of the histological specimen was noted (Figure 2). The basement membrane stained as a dark reddish pink line that clearly demarcated the boundaries of the epithelium with the connective tissue stroma. The cytoplasm stained light pink with a slightly darker cellular outline which made the epithelial cells clearly demarcated. The keratin layer stained prominently with darker reddish pink shade. In the underlying connective tissue stroma, prominently staining collagen fibers, blood vessels and inflammatory cells were noted in bright pink color over a background of faint pink extracellular matrix.

*C) Staining characteristics with synthetic Kala Khatta juice:*

The histological specimen stained non-selectively with a dark-pink to purplish shade. The outline of epithelial cells was prominently stained as dark purple clearly demarcating the cells with a relatively faint pinkish cytoplasm. The basement membrane was not discernably stained, however, the separation between connective tissue and the epithelium could still be easily visualized. The collagen fibers, inflammatory cells and blood vessels with intravascular elements in the underlying connective tissue stroma prominently stained dark purple making them clearly visualizable. Overall, a non-selective purplish staining of the section with discernible epithelium and well-characterized fibrovascular tissue elements was noted.

## Discussion:

Color of the food or a beverage is usually associated with certain flavors and adding them to food enhances the perception of their flavor [7]. For this purpose, edible food coloring dyes have been widely used in food products [8]. Synthetic dyes have advantages of higher color stability, lower microbial contamination, lower production costs and color uniformity [9]. Stains are usually taken up by different tissue components due to various interactive forces such as Coulombic forces, hydrogen bonding and Van der Waal forces. Since the dye molecules are present in clusters within an aqueous solvent, hydrophobic effect which would increase the system entropy leading to general dispersion of the dye molecules and deposition of dye aggregates in the tissue also play a role in the resultant staining caused by these solutions [10].

Synthetic orange juice is one of the most commonly consumed and easily available beverages. These usually contain Carmosine as the colorant imparting its characteristic orange color [11]. Rose syrups contain Anthocyanin dyes, commonly Peonidin, as the coloring agent [12]. Kala khatta, another commonly employed flavor in beverages has been commonly used as an edible dye [13]. An essential component of Kala Khatta flavor is Jamun fruit (Eugenia jambolana) [14]. The fruit consists of yet another pigment. Malvidin, belonging to the Anthocyanidin family, imparting its characteristic purplish color [15]. Additionally, the fruit has been reportedly used as an eco-friendly and economically feasible cytological stain [16].

These dye molecules possess ionic nature which may contribute to the Columbic attraction and therefore, subsequent staining of structures like collagen fibers [17]. The different shades seen in different components of the histological specimen kept in the same staining solution for the same amount of time could be due to difference in rate of uptake by various tissue components and also difference in rate of elimination of the dye molecules when washed in water [1]. The pH of the solutions were measured to be 3.1 for Orange, 3.3 for Rose and 3.6 for Kala Khatta juices. Acidic pH further adds to the long term color stability, particularly of the anthocyanin family of pigments [18].

The prominently staining basement membrane illustrated by using these dyes, could potentially have a role in quick screening of histological specimens without having to use more complex and technique sensitive staining procedures. The bright staining of keratin as presented by out results could have an application in demonstration of keratin pearls or structures of similar composition. The potential of Kala Khatta solution as a stain by its resultant prominent and characteristic staining of the fibrovascular elements cannot be overlooked. A number of lesions such as hemangiomas involve proliferation of such elements and the solution, could be selectively utilized in these situations for efficient diagnosis.

The most important advantage in employing synthetic juices as histological stains is their economic feasibility and eco-friendly composition [19]. The solutions cost a mere amount of Rs. 15 for 250 ml which would be sufficient enough to fill up two Coplin jars used in routine histologic staining. The budget involved is more than a hundred times less than required for procuring the same amount of hematoxylin and eosin, which additionally, have to be imported from the western countries [16]. Apart from cost effectiveness, the solutions could produce comparatively more efficient staining within just five minutes as compared to longer technique sensitive schedules recommended for hematoxylin and eosin. Another advantage is the long shelf life of the solutions, wherein they can effectively be stored under refrigeration for more than a year whilst maintaining their color stability [20].

Our approach, being a novel one, undoubtedly has certain limitations. Firstly, the solutions could not be considered homogenous always and different solutions obtained at different times could have varying concentrations of dyes. Secondly, contaminant debris were observed in histologically stained sections which would warrant requirement of certain filtration procedures. Thus, further research by utilizing these stains in different oral pathologies not only restricted to normal tissues and modifying or refining the solutions is warranted with an objective to develop standardized preparations that could be used definitively and effectively for certain pathologies for an efficient diagnosis. Additionally, histological slides need to be stored in institutions for prolonged periods of time for purposes of teaching or reference. Although our study has demonstrated that these stains are well-preserved without fading over a period of months, further long-term observational studies with regards to stability are warranted in order to analogize with routine standard stains.

## Conclusion:

The results from present study demonstrate that synthetic juices can effectively and efficiently be utilized as makeshift histological stains in case of unavailability of standard ones. The solutions can fairly differentiate various tissue elements and can be utilized for a rapid diagnosis without involving numerous technique sensitive and costly procedures. Ease of availability, cost effectiveness, color stability, long shelf life, environment friendliness are some of the advantages of utilizing these solutions as histological stains. Our research could serve as a pretext for researchers who further wish to venture into this unexplored aspect of histotechnology.

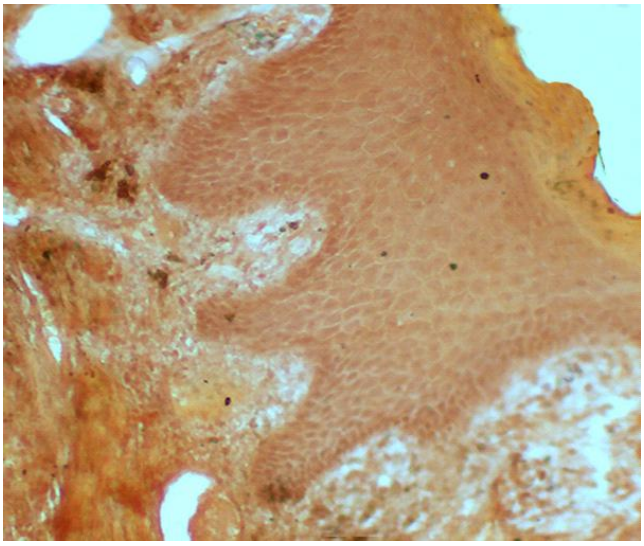


**Figure I:** illustrates staining achieved by Orange solution under 10X Magnification

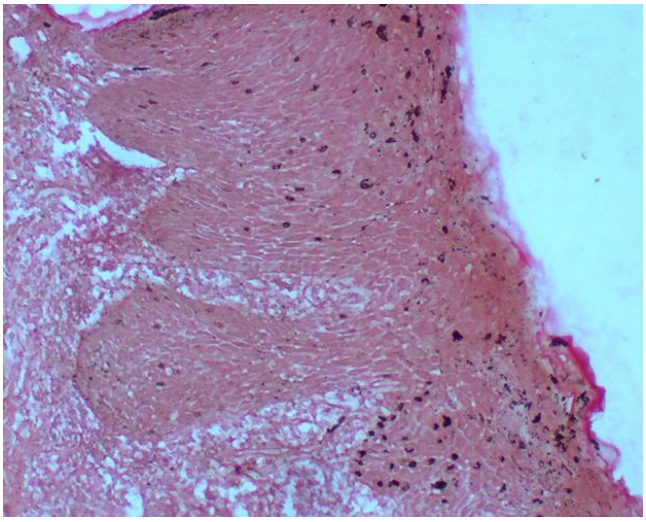
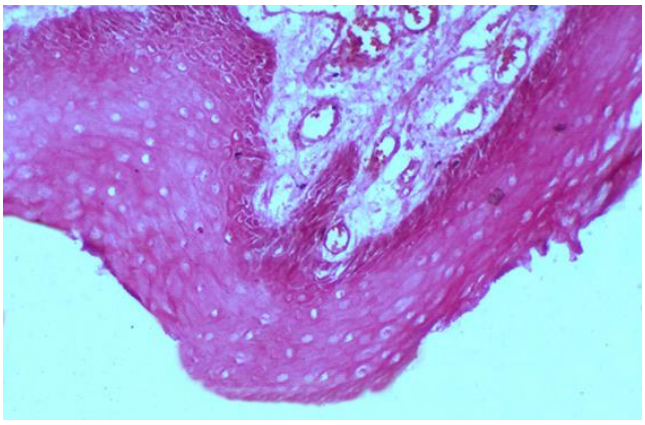
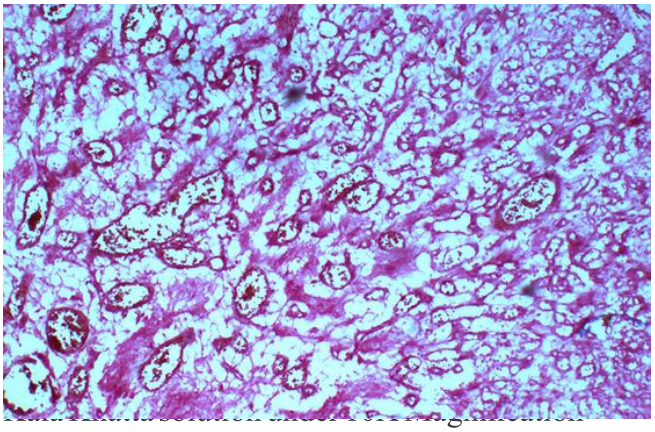


**Figure II:** illustrates staining achieved by Rose solution under 10X Magnification





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