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**COMPARISON OF ROSE PETAL EXTRACT AND HEMATOXYLIN –EOSIN IN
ROUTINE STAINING**

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INTRODUCTION

Natural dyes produce an extraordinary diversity of rich and complex colours as well unexpected results, making them exciting to use. Stains are basically dyes that have been modified for its use in biology. The commonly used hematoxylin stain is obtained from logwood (*Haematoxylon campechianum*) and is the most important and widely used dye in histology, histochemistry, histopathology and cytology [1]. It is used as a primary stain and as a counter stain where it will differentiate acidophilic materials from basophilic materials and to stain non cellular substances such as fibrin, crystals and pigments in various shades depending on the nature of the mordant used and the second stain. Haematoxylin has also been used in the demonstration of several intracellular substances such as mitochondria, chromosomes, chromatin, nucleoli, centrioles, nuclear membrane, ground cytoplasm, cross striations of muscle fibres and chromatin granules in several staining techniques. Haematoxylin is therefore an indispensable dye in histochemistry and histopathology. Hematoxlin stains by oxidation and by the formation of cationic hematein $-Al^{3+}$ molecule. Avwioro, (2011) in his review indicated that Hematoxylin, a natural dye along with Eosin,

a synthetic dye is also extensively used as a counter stain to hematoxylin as it provide a better contrast to the principle stain without hampering the staining of the substrate [2]. The acidic negative charged features binds with the positively charged protein (cationic protein) which include Arginine, lysine and histidine. Until 19th century natural dyes were obtained from various parts of the plant, animals and minerals and were extracted using organic and inorganic solvents. These were used as a primary staining material in various other industries. After industrial revolution a number of synthetic dyes came into existence replacing the natural dyes as they cheaper, brighter, fast and ready to use [3].

The conventional dyes are mostly imported and expensive. On prolonged exposure it causes allergic reaction, irritation to the vital parts such as eyes, skin and nose and some were found to be carcinogenic [4, 5]. In order to overcome this, natural dyes form a better alternative to the synthetic dyes. Some of the commonly used natural dyes are turmeric extract [4] Lawsonia (Henna) [6, 7], rose petals, Hibiscus flowers, and beet root extract [8], Black mulberries (*Morus alba*) [9] and *Morinda lucida* [10].

MATERIALS AND METHODS

- a. Extraction of rose petal stain with ethanol : Rose petals (*Rosa hybrida*) was collected dried, powdered and was immersed in 99% ethanol in the ratio 1:4 and incubated for 24-48 hours for complete extraction of the dye. The extract was purified by 2 stem filtration process. The extract was filtered using cloth mesh and then filtered with Whatman No.1 filter paper. The extracted was then collected and pH was altered upto 4.5 and stored in ependorff bottle at 4 °C until next usage.
- b. Crude extract of rose petal: Fifty grams of rose petals (*Rosa hybrida*) was collected cleaned and was immersed in 100ml of distilled water and incubated for 24-48 hours for complete extraction of the dye. The extract was purified by 2 stem filtration process. The extract was filtered using cloth mesh and then filtered with Whatman No.1 filter paper. The extracted was then collected and pH was altered upto 4.5 and stored in ependorff bottle at 4 °C until next usage.

Paraffin embedded processed tissue slides were obtained from the Department of Oral

Maxillofacial Pathology laboratory for observation and scoring was given from 0 to 3 based on epithelial differentiation, connective tissue differentiation, nuclear staining, cytoplasmic details, staining intensity and contrast.

RESULTS

Crude rose petal extract stained the tissue to a milder intensity where pH altered to 4.5 ethanol rose extract did not impart any color. The duration of the stain was also prolonged for half an hour for both the crude and ethanol rose extract. The observation for ethanol rose extract suggested that there were no staining into the tissue but crude rose extract could stain the tissue. On observing the crude extract rose stain, the epithelium and the connective tissue were not differentiated.

DISCUSSION

Principle of staining suggests that there is an ionic bond between the tissue components and the dye, which is associated with the electrostatic attraction between dissimilar ions. A number of factors are responsible for better staining such as dye concentration, time of action on the solvent, its aqueous or alcoholic nature and most importantly pH of the solvent [9].

With the advent of synthetic dyes and stains and exploitative use in various fields, they

pose a severe threat not only to the environment but also to the human health. Hence, there is an urgent need for replacing the synthetic dyes with the natural products that are environment friendly. A number of plant based products have been identified such as Lawsonia, rose petals, Hibiscus and aloe vera. These products are environment friendly, easy to source and cheap. Raju *et al* (2018) [2] in their study on normal and pathological tissues observed that lawsonia stain as an alternative to synthetic dyes as it gave better results and suggested that this might be due to the similarity of structure of lawsonia and eosin.

Abraham *et al* (2017) [6] on comparing the staining ability of turmeric extract with eosin dye opined that turmeric extract gave statistically comparable result with turmeric extract and concluded that it could be used as an adjunct to eosin dye.

Tousson, E., and Al- Behbehani, B. 2011, [9] experimented with black mulberry (*Morus nigra*) to staining animal tissues and compared the staining ability with beet root extract, hematoxylin and Black mulberry and suggested that black mulberry could be an alternative to synthetic stains such as eosin.

Avwioro *et al* (2005) [10] and Kumar *et al* (2015) [8] opined that pH of the extract determines the ability of the dye to stain

specific tissue structure. Acidic and basic structures are stained by basic and acidic dyes, respectively. By altering the pH of the stain better result could be obtained. Kumar *et al* (2015) on comparing the dyes of rose petal, hibiscus and beet root extract observed that rose petal extract gave better result compared others whereas in the present study rose petal was inferior to Hematoxylin- eosin dye.

From the above it is evident that by suitably adjusting the pH, concentration of the extract, addition of the mordant and the duration of the staining protocol can better the results.

REFERENCES

- [1] Avworo, G. (2011). Histochemical uses of hematoxylin- a review. J Pharm. Clin./Sci. 11: 24-34.
- [2] Raju, L., Nambiar, S., Augustine, D., Sowmiya, S.V., Vanishri, C.H., Babu, A., Rao, R.S. (2018). *Lawsonia inermis* (henna) extract: a possible natural substitute to eosin stain. Open Access. J. of Interdisciplinary Histopathology.
- [3] Bhunyan, R and Saikia C.N. (2005). Isolation of colour components from native dye bearing herbals in Northeastern India. Bioresour. Technol: 96(3): 363-372.

- [4] Ratnas, D., and Padhi B.S. (2012). Pollution due to synthetic dyes toxicity and carcinogenicity studies and remediation. *Int. J. Environ. Sci.* 3(3): 940-955.
- [5] Eom S., Shin, D., and Yoon K. (2001). Improving the dyeing ability of natural colourants on cotton by cationization. *Indian J Fibre Textile Res.* 20(4): 425-431.
- [6] Abraham, M., Nambiar, S., Charagannavar, V., Augustine, D., Sowmia, S.V., Babu, A., Rao, R.S. 2017. Comparison of staining efficacy between turmeric and eosin: A histological study. *J Clinical and diagnostic Res.* 11.ZC05- ZC08. Or DOI: 10.7860/JCDR/2017/26024.10809.
- [7] Kumar, S., Singh, N N., Singh, A., Singh N and Sinha , R K. 2014. Use of *curcuma longa* L extract to stain various tissue samples for histological studies. *Ayu. J.* 35(4): 447-451.
- [8] Kumar, N., Mehul, J., Das, B., Solanki, J.B. (2015). Staining platyhelmenthes by herbal dyes: an eco friendly technique for taxonomists. *Veterinary world:* 8(11); 1321-1325.
- [9] Tousson, E., and Al- Behbehani, B. 2011. Black mulberries (*Morus alba*) as a natural dye for animal tissue staining. *Animal Biology.* 61(1): 49-56.
- [10] Avwioro, O.G., Awoyemi, F.A., Oduola, T. 2005. A novel natural collagen and muscle stain from *Morinda indica* extract. *The Intl. Medical J.* 4(2): 44-48.