

Eco-Friendly Stains

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Abstract

Histopathology is the study of biologic tissues using a microscope to appreciate the diseased cells. It includes many processes from fixation till staining of the tissues using various chemical products. Though natural products are cost effective and non-hazardous, the efficiency and commercial availability of chemical products makes them indispensable. Exposure to the chemicals used in these processes cause various health hazards to the laboratory technicians, pathologists, and scientists working in the laboratory. Hence, there is a dire need to introduce healthy and bio-friendly alternatives in the field. This literature review stress about the natural products and their efficiency to be used as alternatives for chemicals in the histopathology lab.

Keywords: Curcuma Longa Eco-Friendly Stains; Histopathology; Hibiscus Rosasinensis; Lawsonia Inermis; Natural Alternatives; Pterocarpus Osun; Zingiber Officinale.

Introduction

The efficacies of some indigenous herbal dyes for the use in staining are usually non-toxic, eco-friendly and cheap stains for the use in histology.

The extracts from plants are used in various fields in Nigeria and other developing countries. They are used as remedy, pesticides, insecticides, dyes for textile materials as well as stains for biological specimens [1].

Plants have provided an important source of pigments and tannins for the world (Lillie,1969). These plant dyes are found in root, bark, leaves, flowers, stem, fruit skins and nutshells. Some plants may have more than one colour or sources of colour among various parts and or at various stages of their growth and development. These dyes are capable of improving contrast of plant sections so that distinctions can be made between one cell and the other or between cell & its constituents [2].

The application of synthetic dyes have hazardous effect on human health, thus these problems associated with the use of synthetic dyes can be prompted by using dyes of biological origin that will be effective, easy to use, biodegradable and safe to both human and environment.

The most commonly used dye Hematoxylin, which is obtained from a Mexican tree, Hematoxylin campechianum for the use in histopathology. Gair et al (1998) also stressed that herbal stains like saffron, safflower and henna could be utilized for differentiating cells [3].

Therefore, in this present study we will discuss about most commonly available environment friendly stains which includes turmeric, henna, hibiscus and ginger.

Curcuma Longa

The rhizome of *Curcuma longa*, belonging to the family Zingiberaceae, appears morphologically similar to *Zingiber officinale* except for the intense yellow colouring matter.

The dried and ground rhizome of *C. longa* is called turmeric, and it has been widely used as a dye and as a major component in cooking and also used as a pH indicator [4].

The ability of a dye to stain specific tissue structures is determined by certain factors, one of

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which is the acidity of the stain. Acidic structures would be stained by basic dyes while basic structures would be stained by acidic dyes.

Owing to the strong affinity of *C. longa* for the cytoplasm, it can be deduced that the *C. longa* extract dye is acidic in nature because it stained collagen fibres, red blood cells and cytoplasm yellow.

It contained flavanoids, which are typically polyphenolic compounds. Phenols are acidic, due to their ability to release the hydrogen from their hydroxyl group, hence the ability of *C. longa* to stain the basic parts of the cell [5].

When *C. longa* was used as a counterstain for haematoxylin, the nuclei took the blue colouration which enabled a clear contrast to be made between the different structures of the cells.

Zingiber Officinale

The rhizome of *Zingiber officinale* belongs to the family Zingiberaceae, which has a deep yellow colour. The characteristic odour and flavor of rhizome is caused by the mixture of zingerone, shoals, gingerols and volatile oils [6].

This *Zingiber officinale* also contains flavanoids, a polyphenolic compounds, colouring compounds and these phenols are acidic owing to their ability to release the hydrogen from their hydroxyl group [6].

The rhizomes were washed in water to remove sand and other dirt, after which they were peeled off and cut into pieces. These pieces are mixed with 90% alcohol and they are filtered and used [6].

It stained muscle fibers yellow, cytoplasm yellow and nuclei deep green. As it stains basic part of the cells, this shows that reaction of *Z. officinale* stain is similar to the reaction of eosin in Hematoxylin & Eosin technique [6].

Lawsonia Inermis

Henna is a natural dye belongs to *Lawsonia inermis* L., by extraction with clove oil, ethyl alcohol, water and its effectiveness as staining agent for angiospermic stem tissue was studied. *Lawsonia* revealed meta-chromatic property of staining. A 10% w/v crude extract of *Lawsonia inermis* L., leaves in ethyl alcohol and water were used for staining.

Small shrub of henna (*Lawsonia inermis* Linn.) belongs to the family Lythraceae is widely cultivated in tropics and sub-tropics as to enhance beauty, as a dyestuff and elsewhere as a commercial crop [7].

The plant grows at higher temperatures and the major pigment in henna leaf is lawsone (2-hydroxy-1, 4-naphthaquinone), having a fast-dyeing property.

Fresh plants were collected from the fields and the plant of *Lawsonia inermis* was dried in shade for several days at room temperature. The leaves are grinded and powdered mechanically for effective extraction. Dye was extracted with 1%, 5% and 10% solution using solvents, such as water, ethanol and clove oil [7].

It can be used as an effective histological stain without addition of oxidants, mordants and accelerators, used for increasing the intensity of staining.

Cytoplasm of the cell is usually stained with Acidic stains, while the basic stains usually stain the nucleus of the cell (Baker & Silverton, 1976). From this observation it can be estimated that the dye extracted from leaves of *Lawsonia* is acidic in nature [7].

Hibiscus Rosasinensis

Hibiscus is a genus of plants composed over hundred species of *Hibiscus* known for their colorful and showy flowers. *Hibiscus* calyces are rich in anthocyanin, ascorbic acid, and *Hibiscus* acid [8].

The bright color of the *Hibiscus* calyces is by virtue of the presence of anthocyanins. Flower extracts of some species have been used extensively as natural dyes in the textile industry where they are known for their vibrant and long lasting color. There are various studies done on the extract of *H. sabdariffa*, as a histopathological stain and staining fungi and parasites.

Hibiscus calyces were sun dried and powdered. Alcoholic and water extracts were obtained by refluxing the powder with 95% ethanol and distilled water, respectively, for 3 hours, and then cooled and filtered, then used for staining [9].

Staining of the basement membrane showed significant difference when compared with the routine Hematoxylin & Eosin stain [9].

Wing Fruit (*Pterocarpus Osun*)

It is a forest tree that comes under the family of Papilionaceae and gives off a red pigment. Avwioro et al, determined the staining potential of *Pterocarpus osun* extract on tissue sections.

The red pigment was extracted from the powdered stem, with 1liter of 70% ethanol at 78°C for 24 hour. The collagen fibres, red blood cells and muscles were stained using the alcoholic, alkaline and acidic extracts which are again hazardous to the

environment. They concluded that *P. osun* extract is a promising histological stain that can be used for histopathological diagnosis of diseases [4].

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