Application of aqueous plant extracts as Biological stains.

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Abstract— Aqueous extracts from local dye yielding plants: Henna leaves, Madder stem and Flowers of *Hibiscus*, Bougainvillea, Fire flame bush and Madder were used for histological, fungal and Paramecium staining. The acidic cytoplasmic natural stains of Rose and Bougainvillea showed best results for fungal and plant tissue staining, whereas Rose, *Hibiscus* and Henna (instead of Eosin) were foremost for animal histological staining in combination with haematoxyline.

Index Terms— Aqueous plant extracts, Bougainvillea, Biological stains, Henna, *Hibiscus*, Madder.

1 INTRODUCTION

STAINS are generally used to add color to animal tissues, plant tissues, microbes and spores to make them optically distinct and technique is known as staining. Most stains in current use are chemically synthesized from cheap petroleum sources, shows superior fastness properties, are widely available at an economical price and produce wide variety of color [1]. However, they cause skin allergies and other harms to human body on exposure and produce toxic waste, also reduce soil fertility. The use of non-allergic, non-toxic, and eco-friendly stains has become a matter of significant importance due to the increased environmental awareness in order to avoid some hazardous synthetic ones.

Despite the biotechnological advance in medical science today, biological stains are vital in laboratory diagnosis and different staining methods remains an important simple diagnostic tool in diagnostic and research laboratories [2]. Extracts obtained from natural sources such as animal and vegetable sources, plants, insects and soil hold promise as a potential source of cheaper stains. Over 2000 dyes are synthesized from various parts of more than 500 dye-yielding plant species, of which only about 150 have been commercially exploited [1]. In present study, Six local dye yielding plants were chosen to obtain aqueous extracts and their applications in fungal, paramecium staining and histological staining were explored.

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2 METHODOLOGY

2.1 AQUEOUS EXTRACTS PREPARATION

Six local plants: Henna: *Lowsonia inarmis*; *Hibiscus*: *Hibiscus rosa-sinensis*, Madder: *Rubia tictorium* L., Fire flame bush: *Butea monosperma*, Rose: *Rosa indica* Bougainvillea: *Bougainvillea galabra* chosen in this study based on their availability. Aqueous (aq.) extracts of dried powered of Henna leaves, Madder stems and flowers of *Hibiscus*, Fire Flame bush and Rose were prepared by Soxhlet extraction [3]. Staining solutions/ natural stains were prepared using dried powder in water (50mg/ml).

2.2 FUNGAL STAINING

Lactophenol-in-cotton blue is standard stain (acidic pH 3.6) for fungal specimens. A drop of 70% alcohol was placed on a microscope slide and specimen of cottony white fungus was immersed in the drop of alcohol. One/two drops of the Lactophenol-in-cotton blue before the alcohol dries out [1]. pH of the stains was 6-6.5 that was lowered to 4 -5 then used as stains replacing Lactophenol-in-cotton blue. Slides were observed under microscopes and compared.

2.3 PARAMECIUM STAINING

Paramecia were cultured by Hay culture method using pond water as initial inoculum. One drop of paramecium rich culture was placed on clean glass slide and stained using either Methylene blue dye or stains on different slide, observed under the microscope.

2.4 HISTOLOGICAL STAINING

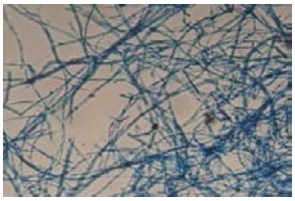
Angiospermic tissue staining: Fresh stem of *Hibiscus* were collected and thin sections of stem tissue were stained using Saffranin and all stains differently. Stained slides were observed under simple light microscope (4x), their staining intensity were observed and compared [4].

Animal tissue staining: Permanent slides of animal tissue sections were obtained by Paraffin embedding method. For staining, slides were dewaxed by dipping in the xylene followed by hydration by a series of alcohols (Absolute to 90% to 70% to 50% to 30% alcohol) then dipped into distilled water for 3 minutes at each step [5]. After rinsing into water the slides were dipped into the Haematoxyline jar for 5 to 10

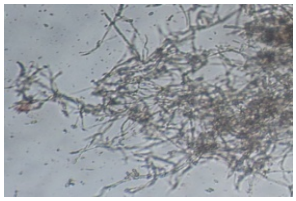
minutes then only rinse with distilled water followed by aq. extract (each separately) for 15 to 20 minutes. Dehydration of the slides was carried out by passing the sections through 30, 50, 70, and 95% alcohol series for few minutes in each strength. Slides stained with only Haematoxyline were further stained by Eosin dye by first dehydration of the slides and then immersion of slide in eosin jar for 3 to 5 minutes. Then wash the excess Stain in 95% alcohol. (which stain cytoplasm of the tissue.) After clearing the slides, the sections were mounted with help of DPX and observation under the microscope [6], [7], [8], [9].

3 Results and Discussion

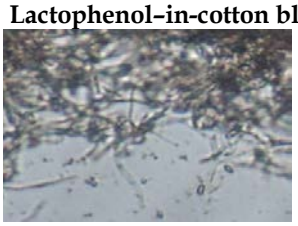
The ability to stain specific tissue structures is determined by the pH values of stain. According to staining theory, acidic structures are stained by basic dyes while basic structures are stained by acidic dyes [3]. The stains treated with acid and base were reported to improve staining potentials for moulds [10]. Hence, in this study, it was necessary to low the pH values of natural stain using dilute HCL. The result of stained white cottony fungus using the natural stains and lactophenol-in-cotton blue is shown in Fig.1. The fungus stained with Bougainvillea and Rose natural stains found to be as efficient as Lactophenol-in-cotton blue in staining. These results are concomitant with work done in 2010 by Briade and his colleagues using extracts of four Nigerian local plants for staining bacteria and moulds [10]. *Hibiscus* and Butea monosperma stains shown good staining compare to Madder and Henna extracts. Application methanolic extracts of *Hibiscus* as biological stain for staining some fungal species was found to be successfully explored by Ihuma et al. (2012) [1]. Madder stain was show clump formation it interfere with the staining and Henna stain shows low capacity to stain the fungal specimen than other ones. Hence both were shows poor staining may be because of their composition of pigments.



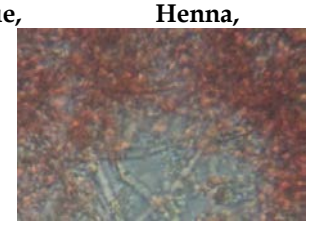
Lactophenol–in-cotton blue,



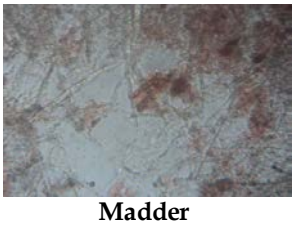
Henna,



Hibiscus

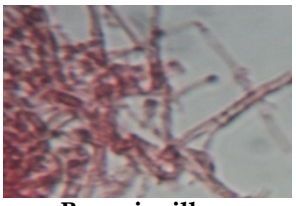


Rose



Madder

Fire flame bush



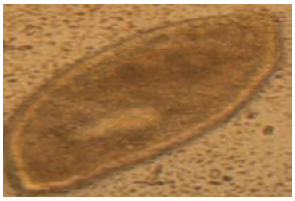
Bougainvillea

Fig.1 Specimens of White cottony fungus were stained with different aq. plant extracts

The potential natural extracts as a staining agent for living cell i.e. Paramecium was investigated in this study. Paramecium staining is useful for study of external morphology, different organelles, behavior and response to various stimuli. It is also used for observe the process of food cycle of paramecium. Figure 2 shows the comparative photographs of stained Paramecium using natural stains and Methylene blue dye. All natural stains stained cytoplasm, cytoplasmic components or cell organelles of paramecium very effectively. Cytoplasm of the cell is usually stained with Acidic stains, while the basic stains usually stain the nucleus of the cell [6]. From this observation it can be estimated that the natural stains were acidic in nature and can replace methylene blue like synthetic stains for paramecium staining. To our knowledge this is the first attempt of paramecium staining using aq. plant extracts as stains.

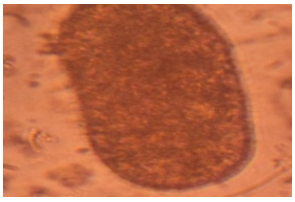
Unstained

Methylene blue



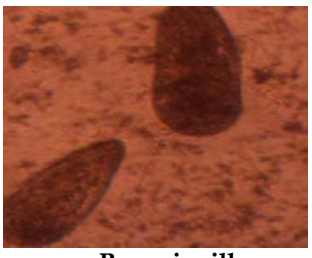
Henna

Hibiscus



Rose

Madder



Bougainvillea

Fire flame bush

Fig.2 Paramecium: unstained/ stained with various stains

The results of histological staining of angiospermic tissue are presented in Fig 3. Henna and Fire flame bush stains were imparted grayish green colour to the sclerenchyma and parenchyma stem tissue. These findings are similar to the work done by Jan et al. (2011) using henna dried leaves extract as angiospermic tissue stain [4]. Bougainvillea natural stain also showed effective staining same as saffranin synthetic dye. Vascular bundles, xylem cells were stained effectively, while cortex and medulla were stained less effectively. Xylem is the water conducting tissue cells therefore; they may be effectively stained with aqueous natural stains. Rose extract showed good staining, whereas as madder and *Hibiscus* extract showed poor staining because they were form clumping with the fluid of stem tissue. Hence, they were not suitable for staining the stem tissue of *Hibiscus* plant. Any previous reports on plants like Fire flame bush and Bougainvillea for plant tissue staining have not been found.

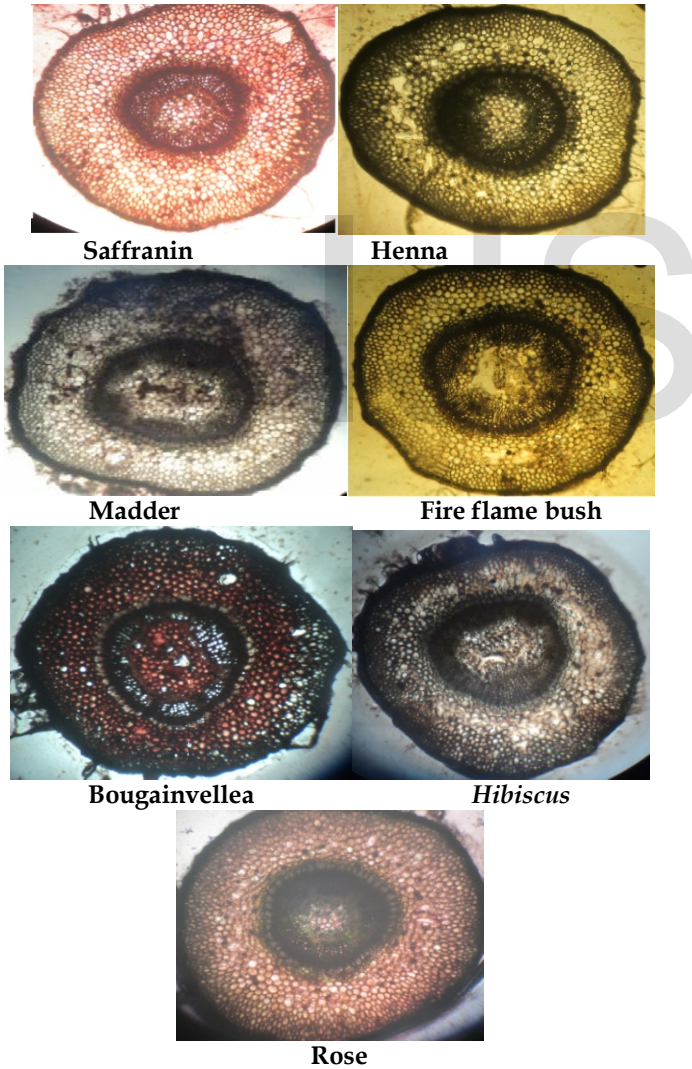
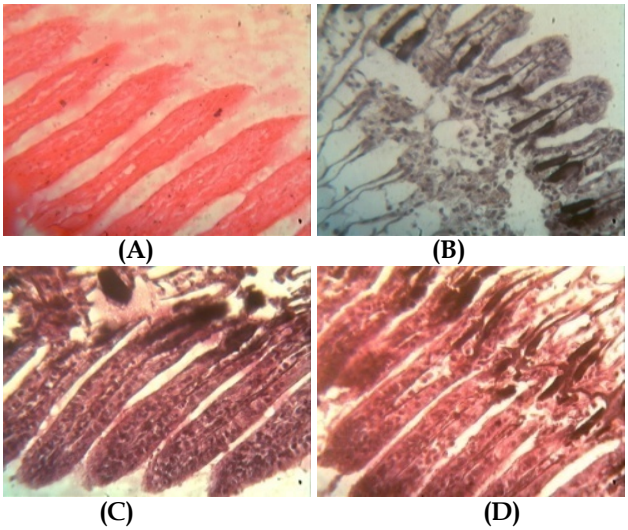


Fig.3: Cross sections of angiospermic stem tissues were stained with different stains

Plant and insect parts have found place in histological staining due to their coloring and dying effects. For instance, plants and insect parts used in histological staining as natural dyes are Haematoxylon campechiaumn, from which haematoxyline is obtained and Dactylopius cacti, from which carmine stain is obtained. The haematoxyline in combination with Eosin which is synthetic dye is used for the demonstration of general tissue structures such as muscle fiber, connective tissue etc. [11]. Haematoxyline is a basic dye that stains acidic components of the cell. Eosin is an acidic dye that stains the basic cytoplasmic components of the cells. A counter stain is a stain with colour contrasting to the principal stain, making the stained structure more easily visible [11]. It is the application to the original stain, usually nuclear stains. Some counter stains which are acidic may lighten or remove the nuclear stains. Nuclei of the cells take the haematoxyline dye and appear dark violet or blue in color, cytoplasm of some epithelial cells; erythrocytes take eosin dye and stain pink [7].

The dyeing of tissues is dependent on binding forces or link to the tissue; or they will simply be rinsed out of the tissue when the section is washed in another reagent [8]. Ionic bonding involves electrostatic attraction between oppositely charged ions is the most important form of bonding in histological staining. Selectivity of staining depends on a sufficiently low stain concentration, on the time of action on the solvent, its aqueous or alcoholic nature and its pH [6]. Eosin is an alcoholic stain and Haematoxyline is aqueous stain extracted from logwood.

According to the results obtained natural stains from Rose, Hibiscus, Henna and Bougainvellea stained cytoplasmic components of the tissue showed better histological staining same as the combination of Haematoxyline with counter stain Eosin. Madder stain and Fire flame bush stain showed poor histological staining (Fig. 4). Histomorphological studies of testis tissue using Curcuma Longa extracts as stains has been reported by Bossey et al. (2012) [6]. Hence, in present study, the aqueous extracts can be called as Cytoplasmic stain which can be used as a counter stain with Haematoxyline instead Eosin.



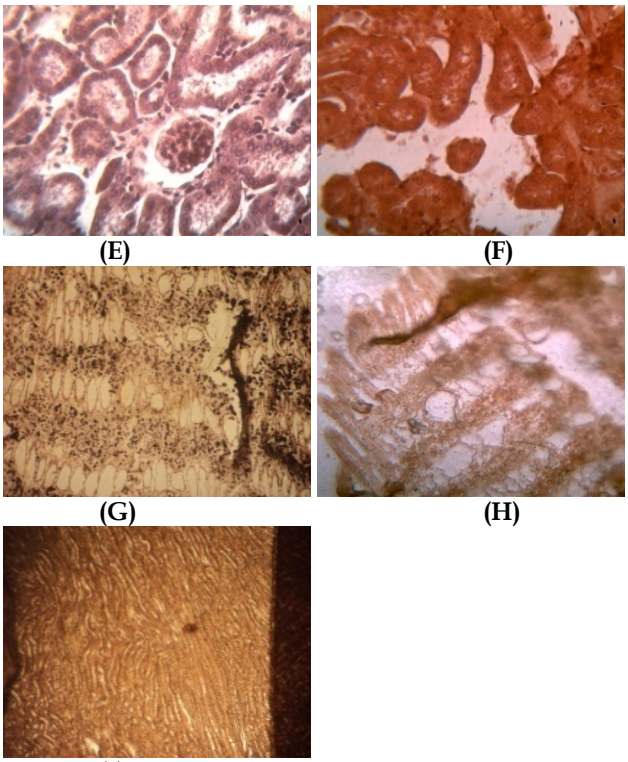


Fig 4 : Histological staining of Animal tissue by various stains

(A)Section of the gills of bioworm stained with Eosin only, (B) Section of the Gills of bioworm stained with Haematoxyline only.(C) Section of the gills of bioworm stained by Haematoxyline with Eosin stain (D)Section of the gills of bioworm stained by Haematoxyline with Rose stain(E) Section of the pancreas tissue of Albino rat stained by Haematoxyline with Hibiscus stain (F) Section of the Pancreas tissue of Albino rat stained by Haematoxyline with Henna stain (G)Section of the gills portion of Bioworm stained by Haematoxyline with Fire flame bush stain (H) Section of the of gills of Bioworm stained by Haematoxyline with Madder stain (I)Section of the Kidney tissue of Albino rat Stained with Haematoxyline with Bougainvillea dye.

4 Conclusion

Six local plants were selected and their aqueous extracts used as eco-friendly and cost effective biological stains. Acidic cytoplasmic natural stains have affinity with basic components of tissue/ fungal species due to which Rose and Bougainvillea extracts stained plant tissue well, may replace saffranin whereas Rose, Hibiscus and Henna worked best in combination with haematoxyline for animal tissues staining instead of Eosin.

References

[1] J. Ihuma, G. Asenge, K. Abioye and S. Dick., "Application of Methanolic Extracts from *Hibiscus Sabdariffa line* As A Biological Staining Agent for Some Fungal Species", *Int. j. plant, animal & env. Sci.*, vol. 2, no. 3, pp. 543-49, 2012.

[2] H. Goodarzian and E. Ekrami, "Extraction of Dye from Madder Plant (*Rubia Tinctorium L.)* and Dyeing of Wool", *World Appl. Sci. J., vol.* 9, no. 4, pp. 434-436, 2010.

[3] O. Chukwu , C. Odu , D. Chukwu , N. Hafiz, V. Chidozie and L. Onyimba, "Application of Extracts of Henna (*Lawsonia inamis)* Leaves As A Counter Stain". *Afri. J. Micro. Res.* Vol. 5 no. 21 pp. 3351-3356, 2011.

[4] H. Jan, Z. Shinwari and A. Khan, "Staining Effect of Dye Extracted from Dry Leaves of *Lawsonia inermis* Linn (Henna) On Angiospermic Stem Tissue", *Pak. J. Bot.*, vol. 43, no. 1, pp. 383-389, 2011.

[5]] S. Iyiola and G. Avwioro, "A New Haematoxyline Stain for The Demonstration of Nuclear and Extra Nuclear Substances", *JPCS,* vol. L, no. 1, pp. 129-131, 2011.

[6] R. Bassey, A. Oremosu and Osinubi, "*Curcuma longa:* Staining Effect on Histomorphology of The Testis". *Macedo. J. Medi. Sci.,* vol.5, no. 1, pp. 26-29, 2012.

[7] E. Tousson and B. Behbehani, "Black Mulberries (*Morus nigra*) As A Natural Dye for Animal Tissues Staining". *Animal Bio.* Vol. 61, pp. 55-58, 2011.

[8] S. Papawee, R. Suppaluk and T. Natthawut, "Extraction of Natural Histological Dye from Black Plum Fruit (*Syzygium cumini*)", *J. Microscopy Soc.Thai.*, vol. 4, no. 1, pp. 13-15, 2011.

[9] Eman, "The Use of Watery Extract of Kujarat Flowers *Hibiscus sabdariffa* As A Natural Histological Stain", *IRAQI J.Medi. Sci.,* vol. 3, pp. 34-40, 2006.

[10] W. Braide, C. Akobundu, R. Nwaoguikpe and Njiribaeko, "The Use of Extracts from Four Local Nigerian Plants for the Staining of Selected Bacteria and Moulds". *Afri. J. Micro.Res.*, vol. 5, no. 1, pp. 79-86, 2010.

[11] M. Wiam and M. L. Sonfada, "*Lawsonia inermis* and *Hibiscus sabdariffa:* Possible Histological Stains", *Trop.Vet.,* vol. 22, no. 1&2, pp. 1-5, 2006.