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Application of Organic Dyes from Roselle calyx (*Hibiscus sabdariffa* linn) for Mycological Staining

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Abstract

Staining with lactophenol and methylene blue is a simple and single step, at the same time it is a convincing method for the microscopic identification of fungal mycelial structures. This study describes the preparation and extraction of natural dye from Roselle (Hibiscus sabdariffa) calyx for single staining techniques of fungal mycelial structure, as well as its staining effect on fungal sporangia. Various treatments using the plant (calyx) aqueous extract pH 1.9 (Temp. 30.1℃), and ethanolic extract pH 2.9 (Temp. 30.1℃), were carried out on different fungal spp, viz: Aspergillus nidulans, Rhizoctonia sp, Rhizopus sp, Penicillium *ctrinum*, *Cladosporium spp* and *Mucor spp*. Different levels of differentiation of the fungal mycelial structures were obtained, using simple staining techniques and viewed under low and high power objectives of Carl zeiss microscope. All the extracts had the affinity to stain fungal mycelia as well as the fungal sporangia, the fungal mycelia and sporangia are stained red and stood out well against fairly red background. The wavelength with highest absorbance was obtained at 460nm for the extracts using scan ultraviolent spectrophotometer model st-uv-755v.

Keywords: *Hibiscus sabdariffa* (Linn), Potassium alum, Staining, Fungal sporangia.

1. Introduction

Calyx is a very important dye-yielding organ of Roselle *Hibiscus sabdariffa Linn* (family Malvaceae). The plant is an annual herb of about 1.5 m tall with reddish stem. The leaves have variable shapes ranging from unlobed to deeply 3-lobed with finely serrated margins. Flowers, mostly unitary, occur in the leaf axils with large pinkish-white petals and reddish base. Calyx red is fleshy 5-valed capsule, covered with sparse hairs. Seeds are dark brown with tufts of microscopic hairs (Smith, 2002). Roselle is native from India to Malaysia, where it is commonly cultivated, and must have been carried at an early date to Africa. It has been widely distributed in the tropics and subtropics hemispheres, and in many areas of the West and Central America has become naturalized Morton (1987). Virtually all dyes were obtained from natural sources, mostly from vegetables plants, trees, and lichens, with a few from insects. Plants dominate as sources of natural dyes, producing different colours like red, yellow, blue, black, brown and a combination of these. Almost all part of the plants like root, bark, leaf, fruit, wood, seed, flower, etc produce dyes (Gulrajani, 1992). The applications of synthetic dyes are presently limited because of their hazardous effects on human and animal health. Bhuyan and Saikia (2004) asserted that the prevalent withdrawal from the use of some of these synthetic dyes is connected with the detection of their hazardous nature. Also most developing countries can no longer afford the ever increasing cost of synthetic dyes (Avwioro *et al.*, 2005). These identified problems associated with the use of synthetic dyes has encouraged the search for low-cost dyes, especially of biological origin, that will be effective, easy to use and safe to both the human and environmental health (Fig. 1). A large number of dyes are obtainable as natural products.

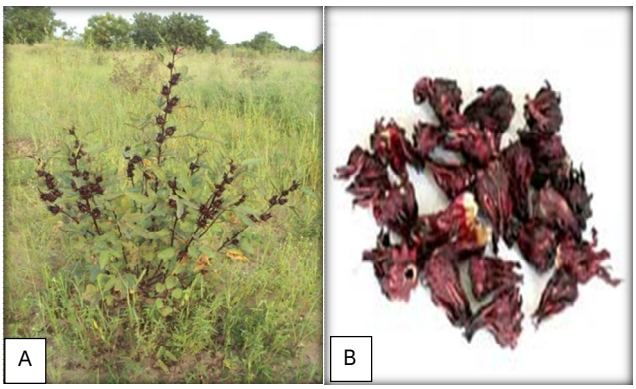

Fig. 1: (A) *Hibiscus sabdariffa* (Linn), (B) Dried Calyx.

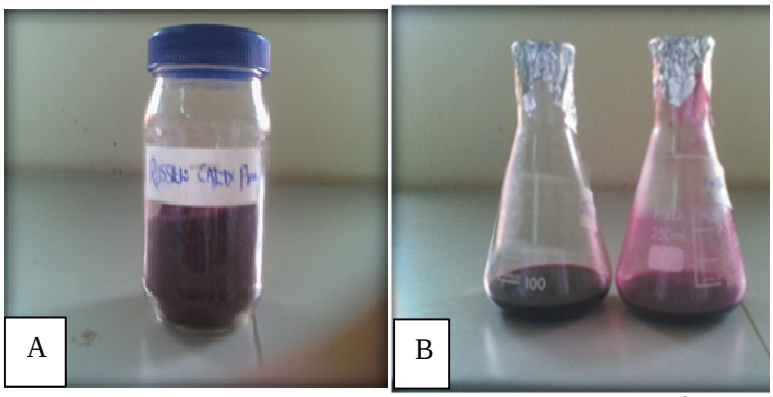

Fig.2: A) Calyx powder, B. Aqueous & Ethanolic extract of roselle

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In Nigeria, many researchers have extracted a number of dyes from a variety of local plants. According to Akpuaka *et al.*, (1998) and Osabohien *et al.*, *(*2002), the local plants - Camwood, Redwood, Henna, Annato, Rothmania, Terminalia, Indiqovine, Kola, Banana, Tumeric, Roselle and Ginger all contain different types of dyes which are used for various purposes. Apart from the use of the extract in mycological staining, the extracts can also be used as a therapeutic, a laxative, an antihypertensive, and a cholesterol lowering medicine (Ali and Wabel, 2005). Also, it exhibits great antioxidant activity, lowers hepatoxicity, reduces fever, diuretic and antiscorbutic in action (McKay and Blumberg, 2006). Although, extracts of Roselle calyx had been reported to have antihypertensive activity (Onyenekwe *et al.*, 1999), protective effect against hepatic oxidative stress (Wang *et al.*, 2000) and many other medicinal and food applications of the extracts, to the best of our knowledge this is the first attempt on the use of aqueous and ethanolic extracts from roselle calyx in mycological staining. In this paper we report the extraction of aqueous and ethanolic natural dye from roselle calyx and its application in mycological staining, with the aim of reducing cost, hazard and environmental unfriendly nature of synthetic dye (Fig. 2).

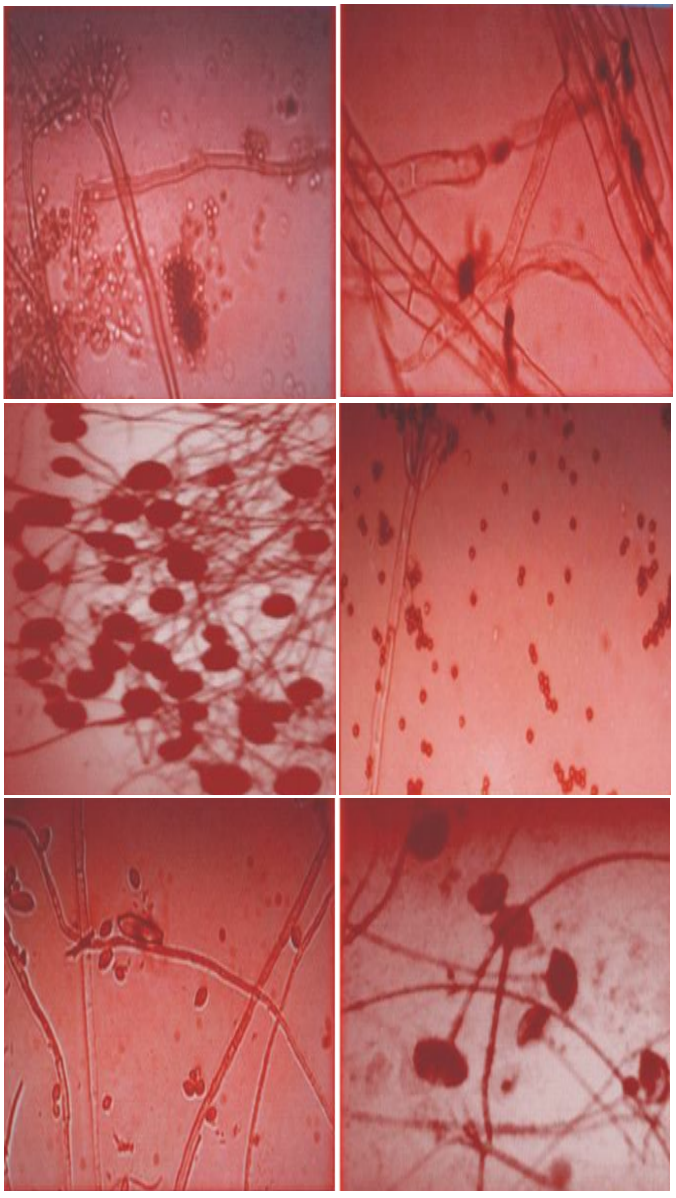

Fig. 3: Fungal species stained with Rossalle extracts (Magnification X400 objectives). (a) *A. nidulans* (b*) Rhizoctonia sp.*(c) *Rhizophus sp.* (d) *P. ctrinum* (e) *Cladosporium sp.* (f) *Mucor sp.*

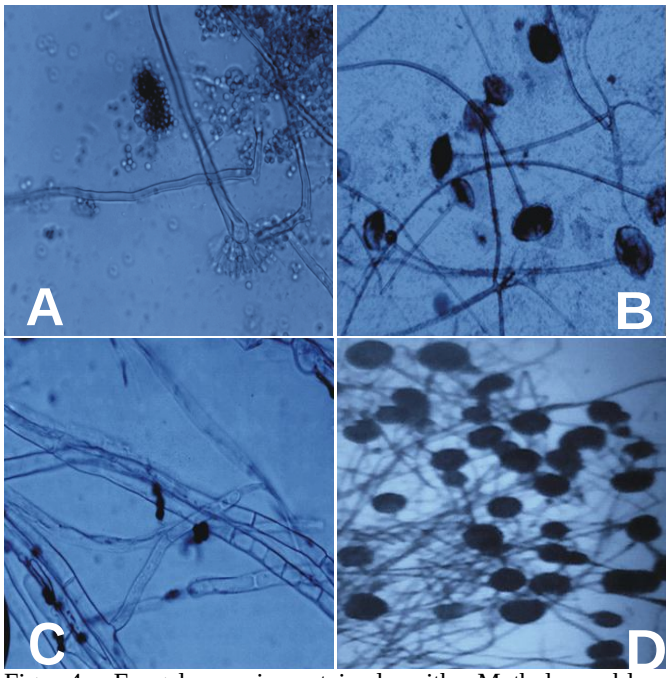

Fig. 4: Fungal species stained with Methylene blue. (Magnification x 400 Objectives) [A] *A. nidulans*, [B] *Mucor sp*, [C] *Rhizoctonia sp.* [D] *Rhizopus sp.*

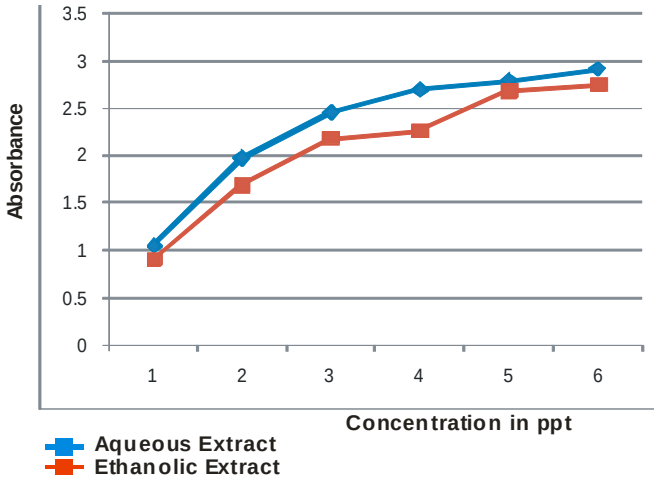

Fig. 5: Photomicrographic absorbance rate of aqueous and ethanolic extracts of *H. sabdariffa* (Linn) red calyx.

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2. Materials and Methods

Preparation of the roselle dye was carried out using the procedure of Egbujo *et al.*, (2008). Dried calyx of roselle was purchased from Gwagwalada (local market) in FCT, and ground to powder using pestle and mortar, then sieved and stored in a dry bottle. Twenty five grams aliquots of roselle calyx powder was weighed and poured into 2 conical flasks, each containing 125mls of water and the other containing 125mls of ethanol (1:5; w/v) Both the aqueous and ethanolic mixture were agitated using Innova 44 rotator shaker at 140rpm for 24hrs.Three grams of potassium alum were added as a mordant, after pH was taken and mixed by shaking vigorously, the two samples were filtered to obtain the colored extract.

2.1 Slide preparation and staining procedure

A drop of roselle extract (dyes) was placed on a cleaned grease free slide, using an inoculating needle. A small piece of fungal mycelia, free of media was removed and transferred to a drop of roselle dyes on slide and by means of the needle the mycelia was teased. The slide was covered with cover slip carefully to avoid air bubbles. The slide was examined first under low power X10, X20 and then under high power X40 of Carl zeiss microscope objectives respectively.

3. Results and Discussion

The staining of the mycelia was carried out in the same manner as the conventional lacto-phenol method as well as that of methylene blue (wet prep). The performance of both the aqueous and ethanolic extracts was quite exciting as shown in (Fig. 3).
From Fig. 3: (a) shows mycelia from 7 days old cultured of *Aspergillus nidulans* shows septate hyphae with wavy conidiophores, conidial heads with short columnar and biseriate. (b) *Rhizoctonia* species shows a clear acute angle of 45^0 from the main hyphae branch (c) *Rhizopus stolonifer* shows a massive net-working hyphe and sporangia (Fig. 4). (d) *P. citrinum* showed single celled conidia with phialide (e) *Cladosporium species* shows septate mycelia with elliptical structure of its conidia (f) *Muco*r species shows sporangiophores which are short, erect and taper towards their apices with short sympodial branches. All the stained fungal mycelia and sporangia took up the calyx dye which made them easier for microscopic identification. The degree of calyx dye uptake by different fungi varies, but different structures such as spores and finger like appearance were stained clearly by roselle dyes extract. Fig. 5 shows a graphical representation of the change in absorbance with concentration at 460nm for the two extracts. Both curves have positive slopes with that of aqueous extract a little higher than that of ethanolic extract. The extracts have the affinity to stain fungal mycelia, sporangia, as well as any fungal external features, the fungal mycelia and sporangia were stained red and stood out well against fairly red background. The results obtained were clearly similar to that of lacto-phenol and methylene blue stain.

4. Conclusion

Red calyx from roselle plants produced an extraordinary red colour which had the affinity for mycological staining and very exciting when used. It will be very satisfying to grow roselle plants as a researcher for the production of dyes from its red calyx, so as to reduce the use of synthetic dyes, since some synthetic dyes have been known to cause some hazards to the environment and human health (Brit, 2008). Also synthetic dyes are increasingly becoming very expensive and are no longer within the reach of many small laboratories in the developing countries. Nevertheless some synthetic dyes are also not environmentally friendly (Siva, 2006). It is for these reasons that an alternative natural dye extracted from Roselle's calyx, which is cheaper and bio-friendly, is being sought as a potential and promising dye for fungal staining. In conclusion, there is need for fine tuning of the extraction procedure, characterization and documentation of dye yielding plants for further development of single and simple mycological stain.

5. Acknowledgement

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