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Full Length Research Paper

Extracts *of Pterocarpus osun* as a histological stain for collagen fibres

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The staining ability of Pterocarpus osun extract on tissue sections was determined. 2 kg of *P. osun* stem was dried, milled to obtain a fine powder and a red pigment extracted from the powder with 1 L of 70% ethanol at 78°C for 24 h. The alcoholic alkaline and acidic extracts were used to stain tissue sections. Collagen fibres, red blood cells and muscles were stained in shades of reddish brown. Preliminary phytochemical screening of this extract revealed that it contained alkaloids, steroid ring, cardiac glycosides and reducing sugar.

Key words: *Pterocarpus osun*, phytochemical screening, tissue sections, stain, mordant.

INTRODUCTION

There are two types of dyes, natural dyes obtained from natural sources and synthetic dyes produced through chemical reactions (Carleton et al., 1976). Some dyes require the addition of mordants, oxidants, accelerators and adjustment of pH before they can stain tissues while others do not require these substances in other for them to stain tissues. That is, simple aqueous or alcoholic solutions of the dyes can be used as stains. These are called simple stains (Avwioro, 2002). Accelerators increase the speed, intensity and specificity of staining, while oxidants convert inactive to active compounds. Mordants act as a bridge between the dye and the tissue (Avwioro, 2002). Natural dyes are widely used in histopathology. In fact the most used dye haematoxylin obtained from the Mexican tree, *Haematoxylon campechianum* is an example of a natural dye (Baker and Silverton, 1976).

Synthetic dyes are very efficient but their applications are limited due to their hazard to human and animal health. This has resulted in the withdrawal of some dyes as their hazards become recognized (Bhuyan and Saikia, 2004). With the worldwide concern favouring the use of eco-friendly and biodegradable materials, the use of natural dyes has once again gained interest (Eom et al., 2001; Padhy and Rathi, 1990; Garg et al., 1991). Most developing countries can no longer afford the ever increasing cost of synthetic dyes. Therefore, the use of naturally occurring dyes from plants, which is less expensive, is being viewed as an alternative to synthetic dyes. Based on the aforementioned, the red pigment extracted from *Pterocarpus osun*, a forest tree that belongs to the family of papilionaceae (Keay, 1989) was investigated as a potential natural dye.

MATERIALS AND METHODS

Preparation of plant extract

The stem of *P. osun* was collected fresh from the Botany department of Obafemi Awolowo University, Ile Ife, Nigeria. The stem was cut into tiny bits of about 0.5 cm thick and dried at 60°C for 5 days in an open air oven (GALLENKAMP). They were milled

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until it became fine powder. 2 kg of the powdered plant material was extracted with 1 L of 70% ethanol under soxhlet at 78°C for 24 h until completion. The extract was filtered and concentrated *in vacuo* at 50°C and finally dried in a descicator to get rid of the residual water. 50 g of the extract was obtained.

Preparation of sections

Six human tissues, 3 mm thick were obtained from the skin, liver, intestine, kidney, lung and spleen at post mortem. They were fixed in 10% formol saline for 24 h and processed for paraffin wax embedding with the automatic tissue processor (SAKURA FINE TECH, Netherlands) by dehydrating through 70%, 90%, 95% and two changes of absolute ethanol for 90 min each. Clearing was achieved through changes of xylene twice for two hours each, infiltrating through two changes of paraffin wax at 70°C and embedded in paraffin wax. Sections were cut at 4 μm with the rotary microtome (SAKURA FINE TECH, Netherlands) and attached to sections and dried at 65°C for 45 min.

Preparation of *P. osun* staining solutions and staining methods

1 g of the dried ethanolic extract of *P. osun* was dissolved in 100 ml each of the following solutions: 70% ethanol, 1% acetic acid in 70% ethanol, 70% ethanol saturated with potassium aluminium alum (mordant) and 1% ammonium hydroxide in 70% ethanol.
Sections were dewaxed in xylene for 4 min and hydrated through 100, 95 and 70% alcohol. Sections were then stained with each of the prepared solutions of *P. osun* for 1 to 30 min. The sections were finally rinsed in water, dehydrated, cleared and mounted in a synthetic mountant.

Preliminary phytochemical screening

The extract of *P. osun* was screened to determine the presence of the following metabolites through preliminary phytochemical screening according to the method of Sofowora (1993). Alkaloids were detected using the Dragendoff's reagent, Mayer's reagent, Wagner's reagent and tannic acid. Anthraquinones were identified using Borntrager's test for free and combined anthraquinones after hydrolysis. Flavonoids were determined by the ferric chloride test, lead acetate test, sodium hydroxide test and ethyl acetate test. Tannin detection was by ferric chloride test and bromine water test, while phlobotannins was with hydrochloric acid. Saponin was determined with the froth tests and haemolytic test, and cardiac glycosides by using the Salwoski's test, Libermann's test and Keller Killiani's test.

RESULTS

Extraction was completed within 24 h. This gave a reddish brown colour, which turned black when it was concentrated *in vacuo* and dried in a descicator to get rid of the residual water. 50 g dry powder was finally obtained.

The sections were compared with heamatoxylin and eosin technique (Figure 1). Staining was observed in solutions of *P. osun* in 70% ethanol (Figure 2), 1% acetic acid in 70% ethanol (Figure 3), 70% ethanol saturated with potassium aluminium alum as the mordant (Figure 4) and 1% ammonium hydroxide in 70% ethanol (Figure 5), but best results were obtained in sections stained with 1% alcoholic solution of *P. osun* (Figure 2) and sections stained with 1% acetic acid in 70% ethanol (Figure 3). It stained collagen fibres, red blood cells and muscle fibres reddish brown within 10 min.

1% (w/v) alcoholic *P. osun* in solutions of 1 % acetic acid (Figure 3) and of ammonium hydroxide (Figure 5) (acidic and alkaline solutions respectively) stained tissue sections reddish brown. The addition of potassium alum as a mordant did not improve the staining qualities of *P. osun* as shown in Figure 4.

Figure 1 (Control). Haematoxylin and eosin technique (skin), X100.

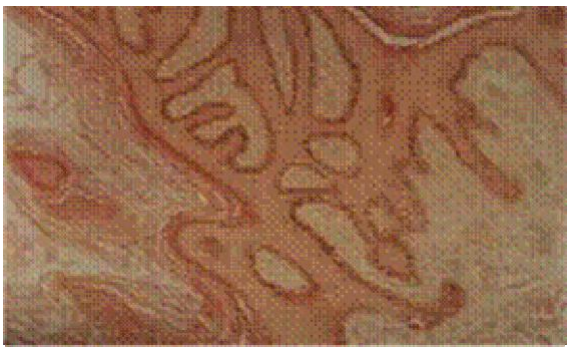

Figure 2. *P. osun* in 70% ethanol (skin), X100.

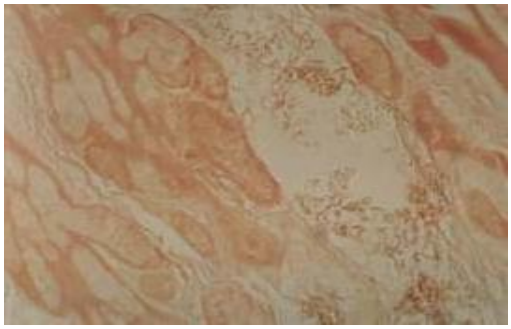

Figure 3. *P. osun* in 1% acetic acid in 70% ethanol (skin), X100.

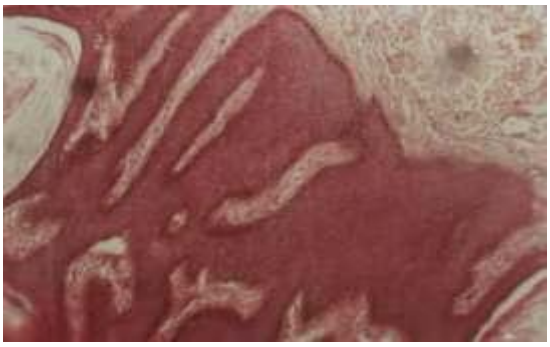

Figure 4. *P. osun* in 70% ethanol saturated with potassium aluminium alum (skin), X100

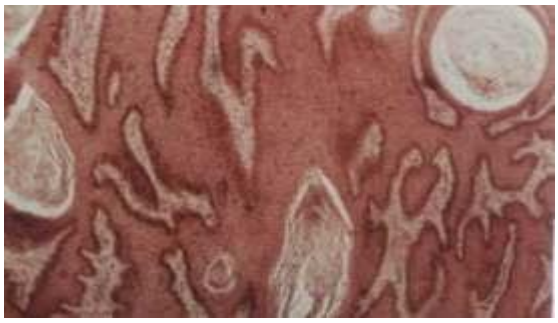

Figure 5. *P. osun* in 1% ammonium hydroxide in 70% ethanol (skin) X100.

DISCUSSION

Cellular structures are selectively stained by various natural and synthetic dyes. Some require combination of stains to demonstrate the presence of some of these tissue structures. Acidity, alkalinity and mordant have been reported to affect some stains. The use of potassium aluminium alum as a mordant had no significant effect on the staining qualities of *P. osun* extract because simple alcoholic solutions stained the tissues, and the addition of a mordant did not improve its staining qualities. This is unlike most dyes used in histochemistry such as haematoxylin, which is first oxidized to haematein and mordanted before it can be used as a stain for tissues. In a previous experiment, Elbadawi (1976) stressed the need for the use of mordants in certain histochemical reactions. He reported that in the Verhoeff's iron haematoxylin stain for elastic fibres, ferric chloride as a mordant was necessary. Hoffman and Bauknecht (1999) had observed that the ionic strength and pH of the staining solutions often affect staining reactions. This however has not been found with *P. osun* extract as it stained at neutral, alkaline and acidic media although with a decrease in the quality of staining in the alkaline region.

There are other natural and synthetic dyes, which do not require addition of acid or base. An example of such a stain is eosin commonly used as a counterstain for haematoxylin. Vickerstaff (1954) stained eosinophils with congo red and stated that the ionic strength influenced the staining reaction between sulphuric acid groups of the dye and the basic groups of the eosinophil granules. Hence Baker and Silverton (1976) concluded that acid tissue elements such as nuclei would have an affinity for a basic stain while cytoplasm, which is basic in character, would have an affinity for acid stain. In view of this, it is expected that *P. osun* is an acid dye. It is therefore concluded that *P. osun* extract is a promising histological stain that can serve as a useful stain for histopathological diagnosis of diseases. Many natural dyes contain not only impurities but also several dye fractions (Banerjee and Mukherjhee 1981). It was, therefore, expected that *P. osun* would contain several components, which can be determined through column chromatography, mass spectophotometry and nuclear magnetic resonance.

REFERENCES

Avwioro OG (2002). Histochemistry and Tissue Pathology. Claverianum Press Ibadan, Nig. pp. 134-213.

Baker FJ, Silverton RE (1976).The theory of staining. Introduction to Medical Laboratory Technology 6th ed. Butterworths, pp. 385 –391.

Banerjee A, Mukherjee AK .(1981) .Chemical aspects of santalin as a histological stain. Stain Technol. 56: 83-85.

*Bhuyan R, Saikia CN (2004). Isolation of colour components from native dye-bearing plants in Northeastern India. Am. J. Pathol. 164 (5): 873-77.

Carleton HM, Drumy RAB, Wallington EA ,Cameron R .(1976). Histological technique. 4th edition. Oxford University Press. Lond. 108nnnnn

Elbadawi A (1976) .Hexachrome modification of Movat's stain. Stain Technol. 51: 249 – 253.

Eom S, Shin D, Yoon K (2001). Improving the dyeability of natural colourants on cotton by cationization. Ind. J.. Fibre Text. Res. 26 (4) : 425-431.

Garg A, Shenda S, Gupta KC (1991) .Effect of mordants on colour of natural dye extracted from tissue flowers (*Butea mongsperma*). Colourage 38 (2): 50-53.

Hoffman EM, Bauknecht N (1999). A dye binding assay for the quantification of solution and cell-bound acidic polysaccharides produced by red algae. Anal. Biochem. 267 (2): 245 – 251.

Keay RWJ (1989). Tress of Nigeria. Oxford Science publications. pp. 50, 85-88, 418, 437.

Padhy RN, Rathi D\ (1990). Effect of mordants on dyeing of cotton with vegetable dyes. Text. Dyer Printer 23 (25): 27-28.

Sofowora A (1993). Medicinal plants and traditional medicine in Africa. Spectrum books 2nd ed. pp. 150 – 153.

Vickerstaff T (1954). The physical chemistry of dyeing, Olive and Boyd, London. p. 352.