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HISTOLOGICAL STAINING PROPERTIES OF *KHAYA SENEGALENSIS* WOOD DUST EXTRACTS: A PRELIMINARY STUDY

Azubuike Nkiruka C*[1], Ikele Ikenna T[2], Onyemelukwe Anulika O[1], Maduakor Uzoamaka C[1], Onwukwe Okechukwu S[1], Maduakor-Oparah Chioma L[3], Chukwu Ikechukwu J[1], Okoye Rita O[4]

[1]Department of Medical Laboratory Sciences, Faculty of Health Sciences & Technology, College of Medicine, University of Nigeria, Nigeria
[2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Nigeria
[3]College of Medicine, University of Lagos Teaching Hospital, Lagos State, Nigeria
[4]Allied Specialist Hospital and Diagnostic Centre, Gwagwalada, Abuja, Nigeria

*Correspondence to Azubuike N.C**.:** Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus. **Tel:** +2348036711136. **E-mail:** nkiruka.azubike@unn.edu.ng

ABSTRACT

Crude aqueous and methanol extracts of *Khaya senegalensis* wood dust were employed as histological stains for the demonstration of general tissue architecture. Preliminary phytochemical screening of the extracts was conducted. Mixtures of the extracts of *K. senegalensis* in acidic, alkaline and neutral solutions were used to stain tissue sections. Preliminary phytochemical screening revealed that *K. senegalensis* extracts contains flavonoids, saponins, tannins, glycosides and reducing sugars. Optimum staining time was 15 minutes for both extracts and they stained the tissues in different shades of red and brown in the various media used. The best and worst stain uptake of tissues was observed with both extracts in alkaline and neutral media respectively. Mordant preparation of the extracts produced no significant staining difference from the non-mordant preparations. Staining solutions of the extracts have better affinity for the cellular cytoplasm and hence gave a good contrast when compared with Hematoxylin and Eosin control sections. In conclusion, *Khaya senegalensis* wood extract is a promising histological stain for the demonstration of general tissue architecture.

Key words: *Khaya senegalensis*, wood dust, stain, histology, mordant

INTRODUCTION

Khaya senegalensis commonly known as African mahogany is a tree belonging to the family Meliaceae. It is a deciduous evergreen tree with height of about 15 to 30m and is native to Cote D'Ivoire, Cameroon, Chad, Nigeria, Ghana, Senegal and other West African countries, though exotically found in Cuba, Australia and India (Von Maydell, 1986). The bark possesses reddish tinged scales and its leaves are compound with 3 – 7 pairs of usually opposite leaflets of about 7 – 12 cm long. It possesses flowers and is a fruit producing tree (Bokkestijn and Francis, 1986).

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According to Hill (1952), it is renowned for its beautiful closely gained hard red-brown wood and the hard timber is utilized for furniture making, high class joining, sculpture, boat building flooring and veneer. Mahogany bark extracts have been used as wood floor stain and occasionally for tanning leather producing a rich mahogany red colour (Lamb, 1966).

Furthermore, extracts of the parts of *K. senegalensis* have a considerable medicinal reputation as a vermifuge, taenicide, laxative, anti-inflammatory, antidiarrheal, antitrypanosomal agent, wound healing agent and disinfectant, depurative and for treating gonorrhoea, malaria and rheumatism (Oliver-Bever, 1960; Atawodi, 2005; Zhang *et al.* 2007; Nwosu *et al.,* 2012; Abdel-Wareth *et al.,* 2014, Orendu *et al.,* 2016). Its roots are also noted for treatment of mental illness, syphilis, leprosy, against sterility and as an aphrodisiac (Vogt, 1995).

In histochemistry, histology and histopathology, the use of dyes aids in the microscopical examination of tissues and cells. Several natural dyes obtained from natural sources such as plants, insects and the soil have been used while the synthetic dyes are obtained through chemical reaction (Carleton, 1976). Hematoxylin, obtained from the log wood *Haematoxylon campechianum* is the most routinely used histological dye for nearly all tissue specimens (Bancroft and Layton, 2013). Other natural dyes from plants that have been shown to stain tissues include extracts of *Hibiscus sabdarifa* leaves, *Pterocarpus osun* leaves, *Morinda lucida* roots, Rhizomes of *Curcuma longa*, *Lawsonia inermis* leaves and *Certonia siliqua* bark (Avwioro *et al.*, 2005a, 2005b and 2007; Azubike *et al,* 2011; Okpidu *et al,* 2012).

Although synthetic dyes are efficient they are not eco-friendly and are becoming expensive; hence they are no longer affordable to many small laboratories and developing countries (Eom *et al.*, 2001). In addition, the limited application and recent withdrawal of some of them is due to the hazard they pose to human and animal health (Bhuyan and Saika, 2004; Alturkistani *et al.,* 2016). With the global acceptance of the use of environmentally friendly and biodegradable material, there has been an increase on research into the discovery of more natural dyes (Eom *et al.,* 2001). *K. senegalensis* extract is used for dyeing fabrics (Nikiema and Pasternak, 2008) but to the best of our knowledge, there is draught of information on its use as a histological dye for tissue sections. In lieu of this, a natural dye extracted from *K. senegalensis* wood dust is being investigated as a possible histological stain in the present study.

MATERIALS AND METHOD

Preparation of the Crude extracts of Khaya senegalenisis wood dust

Aqueous wood extract: Wood flakes from a 4-feet timber of Khaya senegalensis was obtained from a timber dealer at Kenyatta Timber Shade in Enugu metropolis, Enugu State, Nigeria. The flakes were pulverized in a saw dust grinding machine to obtain a fine powder. 500g of K. senegalensis powder was soaked in 800mls of distilled water and the mixture was boiled for 10 mins and left to cool at room temperature. The mixture was filtered with a muslin cloth and subsequently with Whatman No. 1 filter paper. Methanol extract: About 800g of K. senegalensis wood fine powder was subjected to methanol extraction by maceration under gentle agitation for 48 hours at room temperature using 2.5 litres of 75% methanol. A reddish brown coloured filtrate was obtained after sieving with a Muslin cloth and subsequently with Whatmann No. 1 filter paper. The filtrate was concentrated by evaporation of the methanol at room temperature.

Phytochemical analysis

The phytochemical constituents of the aqueous and methanol extracts of Khaya senegalensis were identified by qualitative chemical tests [Trease and Evans, 2002]

Histological processing of the tissue samples for staining

Two English Angora rabbits were euthanized using diethyl ether and the liver, heart, intestine, lungs, kidney, and skin samples of both animals were excised. 3mm thick tissues were obtained from each organ. The tissue samples were fixed in 10% formol saline for 4 days to ensure adequate permeation of the organs in the fixative. The tissues were further processed using the paraffin wax embedding technique for light microscopy with an automatic tissue processor by dehydrating through increasing grades of alcohol, two changes of xylene for clearing of the tissues, and wax infiltration through two changes of paraffin wax at 70^{o}C. The tissues were further embedded in plastic tissue cassettes to give them external support prior to sectioning using molten paraffin wax. Ten (10) sections of 5µm were obtained from each tissue using a rotary microtome [Hertz 150 rotary microtome, Cambridge model]. The sections were floated on water bath, picked with slides (Pyrex quality) and adhered unto the slides by drying at 65^{o}C for 30mins

Preparation of K. senegalensis staining solutions

For the staining solutions of the aqueous extract of *K. senegalensis* [*AEKS*], 100mls each of the following staining solutions were prepared: (i) *AEKS* boiled with 10g of potash alum for 10 mins; (ii) *AEKS* boiled with 10g of caustic potash [potassium hydroxide - KOH] for 10 mins; and (iii) *AEKS* boiled in 10g of KOH for 5 mins, cooled and treated with 0.175 g of potassium permanganate [per 10mls of the solution]

The staining solutions of the methanol extract *K. senegalensis* [*MEKS*] were prepared in different solutions as described by Avwioro *et al.,* (2007). 5 g of the *MEKS* was dissolved in 100 ml of each of the following solutions: (iv) distilled water; (v) 70% ethanol; (vi) 70% ethanol saturated with a mordant, 'potash alum' [aluminium potassium sulfate]; (vii) 1% ammonium hydroxide in 70% ethanol; and (viii) 1% acetic acid in 70% alcohol.

Staining method

180 sections were de-waxed with xylene and taking through descending grades of alcohol and finally into water prior to staining. The staining solutions employed to stain the sections were: *AEKS* alone, solutions (i) to (iii) of *AEKS* and solutions (vi) to (viii) of *MEKS.* Exploratory trials were conducted to determine the optimum staining time for the staining reactions of the *MEKS* and *AEKS* staining solutions. The staining solutions were also used individually as counter stains in replacement of Eosin in the Hematoxylin and Eosin [H and E] staining technique (Baker *et al*, 2001). Control sections for the demonstration of general tissue structure were obtained by the H and E staining technique.

RESULTS

Phytochemical screening of Khaya senegalensis wood dust

The wood dust extracts of *KS* contained flavonoids, saponins, tannins and glycosides and reducing sugars [Table 1]. *Staining reactions of the Crude MEKS and AEKS*

The optimum staining for both extracts was about 15 mins. Figure 1 (I–VII) are photomicrographs of tissue sections stained with *K. senegalensis* extracts. Both extracts stained the acid and basic components of the tissues though a stronger affinity for the basic components of the tissue (cytoplasmic structure) was observed. Very poor tissue colour uptake was obtained after staining with preparation of *MEKS* in distilled water (data not

shown) but a satisfactory stain uptake when dissolved in 70% ethanol was observed (Figure 1-III).

Table 1: **Phytochemical screening of *MEKS and AEKS***

CONSTITUENTS	*AEKS*.	*MEKS*
Flavonoids	+++	++
Alkaloids	-	-
Saponins	+++	+++
Tannins	++	+++
Glycosides	++	+++
Steroids	-	-
Reducing sugars	+++	+++

Key: -: Absent; +: Trace; ++: Moderate; +++: Abundant; *AEKS*: Aqueous Extract; *MEKS*: Methanol Extract

Table 2: Various staining solutions of *Khaya senegalensis* extracts and colour uptake by tissues

Type of extract	Staining solutions used	Tissue colour uptake
Methanol extract	1% Acetic acid in 70% alcohol as a counter stain for Hematoxylin	Pinkish brown
	1% ammonium hydroxide in 70% alcohol as a counter stain for Hematoxylin	Deep brown
	70% alcohol as counter stain for Hematoxylin	Pinkish-red
Aqueous extract	Boiled with potash alum and used as a counter stain	Light brown
	Boiled with caustic potash only	Red
	Treatment with potassium per manganate	Yellowish brown

MEKS in acidic medium produced a satisfactory staining (Figure 1–VI) after staining tissue with hematoxylin. In alkaline medium [1% ammonium hydroxide in 70% ethanol] the *MEKS* produced an enhanced stain uptake though with fairly moderate definition and differentiation of cellular structures (Figure 1–IV). Mordanting did not produce any obvious difference in staining ability of the extracts.

Staining solution of *AEKS* mixed with potash alum did not produce satisfactory staining effect as a faint colour uptake was observed. However, *AEKS* prepared with caustic potash [alkaline media] produced a good, enhanced and well-defined staining effect when used alone ((Figures 1–I) whereas, when used as a counter stain for hematoxylin, it produced a weaker staining effect (Figures 1–V and 1–VII). Addition of potassium permanganate to *AEKS* produced an intense yellowish-brown colouration within 60 seconds (Figure 1–II). A summary of the colour uptake of the various staining solutions of the extracts used is shown in Table 2.

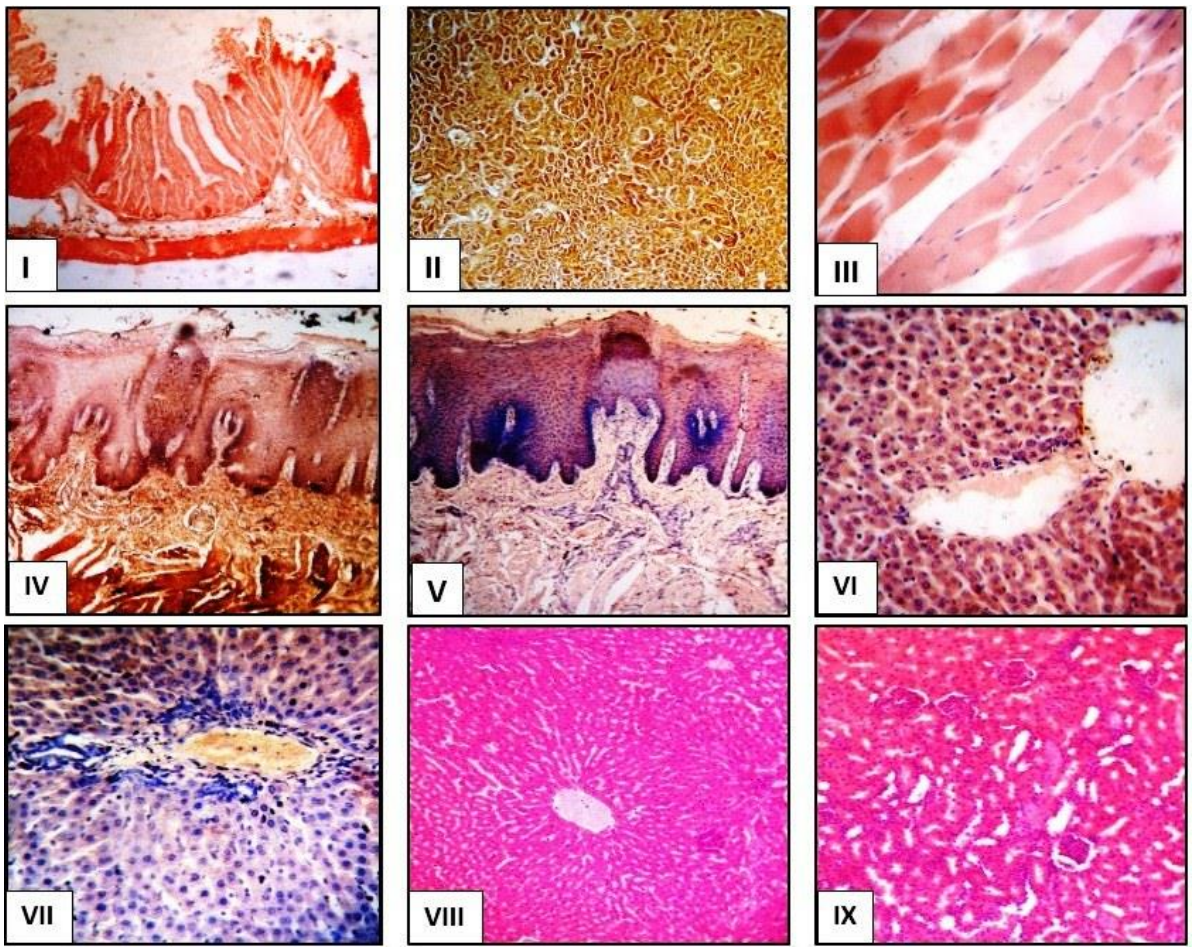

Figure 1 [I-IX]: Photomicrographs of tissue sections stained with the extracts of *Khaya senegalensis*. **I**: Jejunum section stained with *AEKS* boiled with caustic potash [KOH]; **II**: Kidney section stained with *AEKS* boiled with KOH and treatment with potassium per manganate used in replacement of Eosin in the H&E technique; **III** : Smooth muscle section stained with *MEKS* in 70% ethanol; **IV**: Skin section stained with *MEKS* in 1% ammonium hydroxide in 70% ethanol; **V**: Skin section stained with *AEKS* boiled with KOH used in replacement of Eosin in the H&E technique; **VI**: Liver section stained with *MEKS* in 1% acetic acid in 70% ethanol used in replacement of Eosin in H&E technique; **VII**: Liver section stained with *AEKS* boiled with KOH used in replacement of Eosin in the H&E staining technique; **VIII & IX**: Control H&E sections of Liver and Kidney tissues respectively.

DISCUSSION

The present study has shown the staining ability of the wood extracts of *KS.* The major phytochemical constituents in both extracts of *KS* were found to be flavonoids, saponins and tannins, however, it is not clear as to which of these compounds contains the active staining principle.

The extracts from *KS* wood dust was observed to have stained the basic and acidic components of the tissues, but a stronger affinity for the basic structures was noted. Dyes with an affinity for the basic structures of a histological specimen are termed acidic dyes (Ochei and Kolhaktar, 2000; Baker *et al,* 2001). Two phytochemicals, flavonoids and tannins, found in rich quantities in *KS* wood extracts, are the most natural phenolic compounds. The dyeing property of a plant extract correlates the appreciable amounts of these phytochemicals in

the plant (Jondiko and Pattenden, 1989; Shadid *et al*., 2009). Polyphenolic compounds and phenols are acidic in character because they lose a positive hydrogen ion (H^+) from their hydroxyl group (Kumar *et al*., 2014**)**. Hence, in dye-tissue reactions, an electrostatic attraction of unlike ions exists whereby the anions of an acidic dye interacts with tissue structures that are rich in cations (Prento, 2009). Plausibly, it may be inferred that the stain from *KS* wood extract is acidic in nature due to its strong affinity for the basic components of the tissue.

Dyes are able to stain specific tissue structures due to some factors such as pH, ionic strength and mordanting (Hoffman and Bauknecht, 1999). In this study, simple aqueous and alcohol solution of both extracts especially *MEKS* produced very faint and unsatisfactory staining results. Better staining effects were achieved by altering the pH (both acidic and alkaline regions) than when used in neutral media. At low pH, a satisfactory staining result was obtained, however at high pH fostered by ammonium hydroxide and caustic potash [KOH], intense staining reactions were achieved, although less definition and differentiation of the cellular structures were observed with the use of ammonium hydroxide. These findings connote that the staining component(s) present in *KS* may be favoured by either a low pH or a high pH leading to improved staining quality. Avwioro *et al.* (2005a), however, reported that some natural and synthetic dyes do not require the addition of a base or an acid.

A mordant is a metallic salt that acts as a bridge between the tissue and stain, hence enabling stain uptake (Ochei and Kolhaktar, 2000, Hunger, 2007). The use of mordants in the present study produced no significant effect on the staining quality of both extracts as there was no difference between the staining reaction of the mordant and non-mordant preparation of this extract. This agreed with Avwioro *et al.,* (2005b) who reported that mordants had no significant effect on the staining quality of some extracts. More so, several dyes such as eosin, neutral red and methylene blue do not require mordants before they can be used as stains (Baker *et al.,* 2001; Avwioro, 2002). Contrarily, Hematoxylin, a natural histochemical and histological dye requires a mordant prior to staining (Bancroft and Layton, 2013).

It was observed that treatment with potassium permanganate was not necessary in enhancing the staining Fability of the extract but rather decolourized it from the colour it imparts on the tissue to a yellowish brown colour. It is well known that the staining property of Hematoxylin, haematin, may be obtained after oxidation by chemical agents [such as potassium permanganate and sodium iodate], which is termed a chemical ripening process (Bancroft and Layton, 2013), whereby the pale yellow-brown colour of hematoxylin is changed to deep mahogany brown after the oxidation. However, the effect observed with potassium permanganate treatment in the present study is not well understood. Further studies to elucidate this is required.

In conclusion *Khaya senegalensis* is a promising histological stain which is natural and readily available. The dye may also serve as a useful cytoplasmic stain to replace eosin in the Hematoxylin and eosin technique for histological diagnosis of diseases and demonstration of the normal histological architecture of tissues.

CONFLICT OF INTEREST: None

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