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**Histomorphological Assessment of the Alcoholic Extract of *Saccharum officinarum* Bark as a Primary Stain**Mohammed, O.M.\*<sup>1</sup>, Alhassan, M.<sup>1</sup>, Muhammad, A.T.<sup>1</sup>, Mohammed, I.<sup>1</sup>, Abubakar, U.<sup>1</sup>, Salihu, A.<sup>2</sup>, Umar, A.<sup>1</sup>, Bello, M.<sup>2</sup> and Isah, A.Y.<sup>2</sup>Department of Histopathology, School of Medical Laboratory Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto <sup>1</sup>, Department of Morbid Anatomy and Forensic Medicine, College of Health Sciences, Usmanu Danfodiyo University, Sokoto <sup>2</sup>.

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<https://dx.doi.org/10.4314/sokjmls.v9i2.7>**Abstract**

*Saccharum officinarum* (Sugarcane) refers to several species and hybrids of tall perennial grass in the genus *Saccharum*, tribe *Andropogoneae* and belong to the grass family. It is consumed directly in confectionery, used to sweeten beverages, as a preservative, as a decorative finish for cakes and as a raw material in the food industry. The aim of this study was to evaluate the histomorphological assessment of the alcoholic extract of the *Saccharum officinarum* bark as a primary stain. The *Saccharum officinarum* barks were dried in shade for 1 week. The dried stalk was grinded into powder with pestle and mortar. The various alcoholic concentrations 5%, 10% and 15% with different mordant (potassium alum and ferric chloride) were constituted and used to stain liver and kidney sections. Five percent (5%) alcoholic extract of *Saccharum officinarum* bark mordanted with potassium alum stained the liver and kidney tissue section in the shade of pale pinkish and pale red while 10% and 15% of alcoholic extract of *Saccharum officinarum* bark mordanted with ferric chloride as a mordant stained the tissues pale red, with excellent and well-preserved tissue constituents. In conclusion, the data obtained from the present study suggest that the alcoholic extract of *Saccharum officinarum* bark can be used as a substitute for haematoxylin due to its domestic availability.

**Keywords:** Histomorphological, Extract, *Saccharum, officinarum*, Bark, Stain.

**Introduction**

*Saccharum officinarum* is a tall perennial tropical grass, which tillers at the base to produce unbranched stems from 2 to 4 m or more tall, and to around 5cm in diameter. It is cultivated for

these thick stems, stalks or canes, from which the sugar is extracted (Baldick, 2013; Paterson *et al.*, 2012). The plant grows in temperate tropical regions of India, Southeast Asia, and New Guinea (Daniels, 1999). The plant propagation is achieved by both sexual and asexual methods. The plant produces seeds which can be used for propagation but mostly used for the development of hybrids. But modern commercial *Saccharum officinarum* cultivation follows vegetative or asexual propagation through stem cuttings. The most common practice today is to use setts of *Saccharum officinarum* stalk which may have 2 to 3 buds, but longer setts with 4 to 6 buds or smaller single budded setts are also being used for planting sugarcane (Baldick, 2013). The plant is also grown for biofuel production, especially in Brazil, as the canes can be used directly to produce ethyl alcohol (D'Hont *et al.*, 1996). The plant is cultivated throughout the world for its sugar-rich stalks (Mukherjee, 2000). It is consumed directly in confectionery, used to sweeten beverages, as a preservative in jams and preserves, as a decorative finish for cakes and patisserie, as a raw material in the food industry, or fermented to produce ethanol (Gulrajani, 2001). It is one of the most efficient photosynthesizers in the plant kingdom. Stains are prepared from dye which have been manufactured to rigid specification or have been subjected to rigid quality assurance procedure to ensure that they are suitable for this specialized purpose. (Baker and Silverton, 2001). Stain is also referred to as dye which has affinity to a particular part of cell (Ochei and Kolhatkar, 2000). Tissue and their constituent cells are usually transparent and colourless when

examined under the light microscope with little or no differentiation of the various structure (Ochie and Kolhatkar, 2007) Dye are either obtained from either natural or synthetics sources. Natural colour is obtained from plant and animal sources while synthetic dyes are obtained from chemical substances (Grace *et al.*, 2014). Despite the biotechnological advance in medical science today, biological stains are vital in laboratory diagnosis and different staining methods remain an important simple diagnostic tool in diagnostic and research laboratory (Goodazian *et al.*, 2010). Natural stains are dyes that have been derived from plant and animal sources. Historically, plants have been used for the extraction of majority of dyes. Various plant parts include the root, seed, leaves, heartwood, bark, wood, shaving, flowers, fruit, rinds, and hulls serve as a natural dye sources. The famous blue dye, indigo, is obtained from the leaves of the plant *Indigo feratinctoria*. Haematoxylin is a natural dye that is extracted from the core or heartwood of the tree *Haematoxylin campechianum*. One of the few vegetables dyes in histology is the orcein. It is extracted from lichens by the action of ammonia and air. It is a violet colour, soluble in alkalis and weak acids. It is employed in the Taenzer-Unna orcein method for the demonstration of elastic fibres. Like the orcein, litmus is extracted from lichens and is exposed to ammonia and air but in addition, it is treated with lime and potash or soda. Litmus was greatly used as an indicator but is now superseded by synthetic brands. Saffron as a dye has only one use in histology. It has been incorporated by Masson into connective tissue stains; it is extracted from the plant, *Crocus sativus*. One of the oldest in histology is cochineal. It is a dye extracted from the bodies of female cochineal insect. When obtained, the dye is treated with alum and the result is a product relatively free from extraneous matter and is known as carmine (Ochei and Kolhaktar, 2007). The commonest staining method in histopathology is the Haematoxylin and Eosin (H and E) staining method. Haematoxylin is a foreign stain as such it is expensive and not easy to come by (Ihuma *et al.*, 2012) some chemically synthesized stains are toxic to human environment and therefore staining of various biological tissue by the application of natural

dyes would be more cost effective and reduces their effect on human environment (Avwioro, 2014). Also, some dye components are carcinogenic or at least strongly allergic resulting in their withdrawal as their hazard becomes recognized (Blender 2002, Bhuyan and Saiki, 2004). Development of new histological staining method remains justified, especially if the new stain is harmless, easy to use, commercially favorable, cheap and gives the practical result (Penney *et al* 2002). The aim of the study was to evaluate the staining capability of *Saccharum officinarum* bark extract on different histological samples.

## Materials and Method

### Study Location:

The study was carried out in Histopathology Department, Faculty of Medical Laboratory Science, Department of Pharmacognosy and Ethno Pharmacy, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto.

### Experimental Animals

One (1) experimental Wistar rat was used for the research, which was purchased and kept in the animal house of faculty of Pharmaceutical Science, UsmanuDanfodiyo University Sokoto.

### Animal Sacrifice

The Wistar rat was anaesthetized using chloroform vapour. The liver and kidney were harvested, washed with normal saline and transferred into 10% formalin fixative container.

### Procurement of *Saccharum officinarum*

The *Saccharum officinarum* was purchased at Kasuwar Daji in Sokoto North Local Government, Sokoto State. The barks were scraped using a knife and dried under the sun for two (2) days. The dried bark was then grounded into fine powder using pestol and mortar.

### Plant Identification

The plant identification was conducted at the herbarium unit of Department of Pharmacognosy and Ethanopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. And the specimen Voucher Number: PCG/UDUS/POAC/0010 was assigned and deposited in the herbarium unit of the same department.

### **Extraction Procedure Using Ethanol**

The *Saccharum officinarum* bark was grounded into fine powder. And the powder was weighed into different grams (5g, 10 and 15g) and poured into their respective labeled beakers. To each beaker, 100ml of ethanol was measured and poured into the beaker and the mixtures were stirred with the aid of an applicator. The lids of the beakers were covered and allowed to stay for 24h at room temperature. The mixtures were sieved using a white cloth material, and then filtered with a filter paper. Various mordants were then added.

### **Ethanollic Extraction and Potassium Alum as Mordant**

The fine filtered extracts (5g, 10g and 15g) were mordanted with potassium alum until it becomes saturated and then used for the staining of the tissue sections.

### **Ethanollic Extraction and Ferric Chloride as Mordant**

The fine filtered extracts (5g, 10g and 15g) were mordanted with ferric chloride until it become saturated and then used for the staining of the tissue sections, Slide Preparation and Staining

The kidney and liver tissues were processed for histological analysis using an automatic tissue processor. The tissues were embedded, sectioned with rotary microtome to produced 5micro liter thickness of the sections. The sections were floated out in warm water bath, picked onto the albumenized microscopic glass slides and fixed on the hot plate.

### **Procedure for Haematoxylin and Eosin Staining Technique**

The Sections were dewaxed in 3 changes of xylene, 1, 2, 3, for 5 minutes each. Then hydrated in descending grades of alcohol, absolute, 95% and 75% for a minute each and then to water. The sections were stained in Harris haematoxylin for ten minutes, rinsed in water and differentiated in 1% acid alcohol for few seconds. The sections were then rinsed in water, counterstained with eosin for 2 minutes and dehydrated in ascending grades of alcohol, 70%, 95% and absolute alcohol. The sections were finally cleared in xylene, air dried for few minutes and mounted with DPX (Ochei and Kolhatkar, 2007).

### **Procedure for *Saccharum officinarum* Extract Mordanted with Potassium Alum and Eosin Staining Technique**

The Sections were dewaxed in 3 changes of xylene, 1, 2, 3, for 5 minutes each, hydrated in descending grades of alcohol, absolute, 95% and 75% for a minute each and then to water. The sections were stained in the extract mordanted with potassium alum on the various concentration (5g, 10g and 15g) of *Saccharum officinarum* extract and stained for different time duration (20minutes, 15minute and 10minute) respectively. The sections were rinsed in water, differentiated in 1% acid alcohol for few seconds and rinsed in water. The sections were counterstained with eosin for 2 minutes and dehydrated in ascending grades of alcohol, 70%, 95% and absolute alcohol. The sections were finally cleared in xylene, air dried for few minute and mounted with DPX.

### **Procedure for *Saccharum officinarum* Extract Mordanted with Ferric Chloride and Eosin Staining Technique**

The Sections were dewaxed in 3 changes of xylene, 1, 2, 3, for 5 minutes each, hydrated in descending grades of alcohol, absolute, 95% and 75% for a minute each and then to water and stained in the extract mordanted with ferric chloride on the various concentration (5g, 10g and 15g) of *Saccharum officinarum* extract and stained for different time duration (20m, 15m and 10m) respectively. The sections were rinsed in water, differentiated in 1% acid alcohol for few seconds and rinsed in water. The sections were counterstained with eosin for 2 minutes, dehydrated in ascending grades of alcohol, 70%, 95% and absolute alcohol and cleared in xylene. The sections were air dried for a few minutes and mounted with DPX.

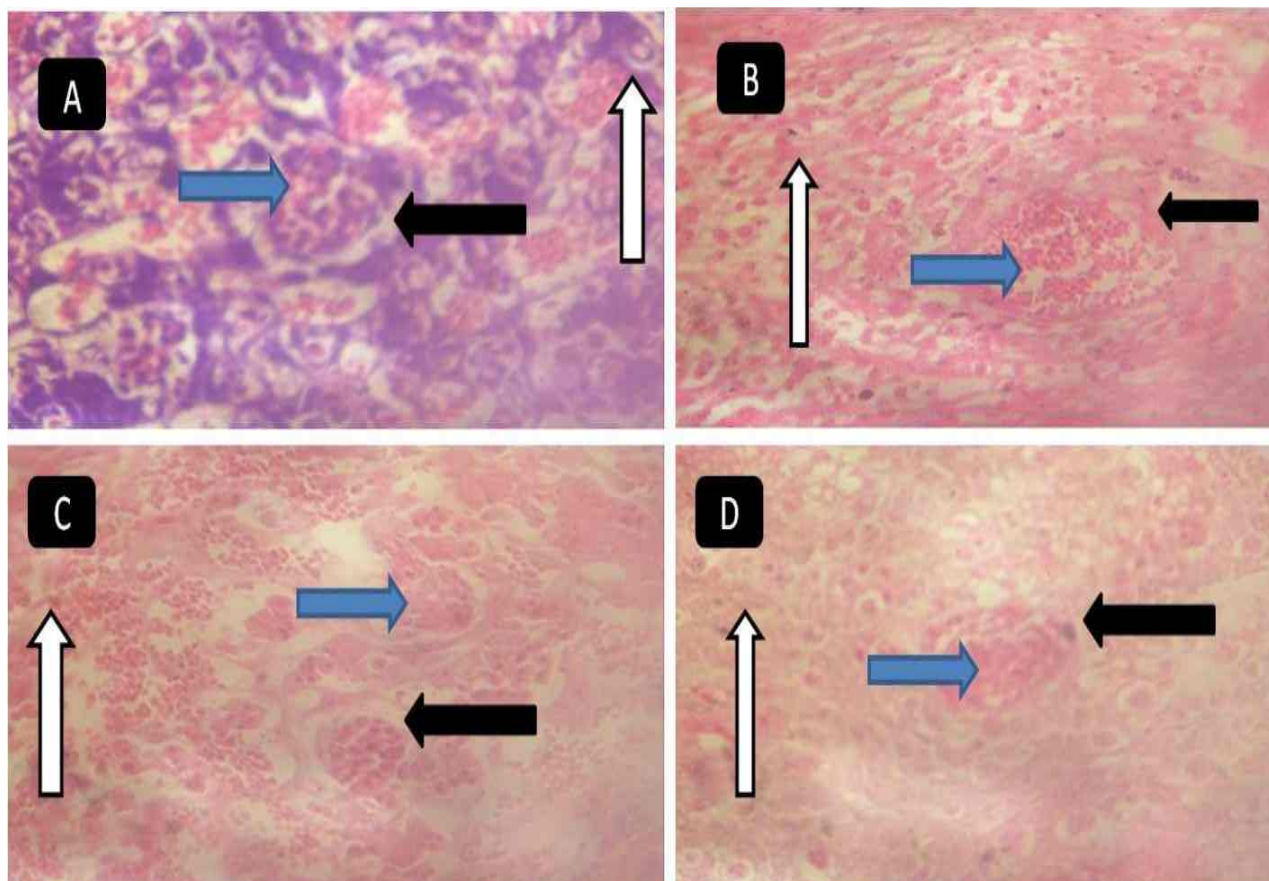
**Table 1 Summary of the Study Design**

Mixture of <i>Saccharum officinarum</i> extract with mordant	pH value	Duration of staining	Colour
5% extract with potassium alum	4.7	20 minutes	Dark purple
5% extract with ferric chloride	2.3	20 minutes	Dark green
10% extract with potassium alum	4.0	15 minutes	Dark purple
10% extract with ferric chloride	2.0	15 minutes	Dark green
15% extract with potassium alum	3.7	10 minutes	Dark purple
15% extract with ferric chloride	2.1	10 minutes	Dark green

**Results**

Complete extraction of *Saccharum officinarum* bark gave a light purple colour. The addition of mordant changed the colour of the extract to dark purple and dark green colour respectively. With the addition of ferric chloride as mordant the extract turned to dark green colour, while the addition of potassium alum as mordant the extract turns to dark purple colour. Various solutions were prepared from the ethanolic extract stained sections of liver and kidney with different time and pH. Various ethanolic extract modifications at varying concentrations of 5%, 10%, 15%, and under varying pH (2-4) were used to stain some selected organs at different interval of 10 minutes, 15 minutes and 20 minutes, the selected organs were able to give a satisfactory demonstration of the general tissue structure. The stained slide were examined to determine the staining pattern of *Saccharum officinarum* bark extract with addition of a mordant. With regard to the conducted study, the result of histopathological sections stained with *Saccharum officinarum* bark extract solutions in plate 1 to 4, all stained the cytoplasmic components pale pinkish depending on the concentration of the solution. The cytoplasm of the kidney stained in shades of pinkish and the nucleus-stained shades purplish. The

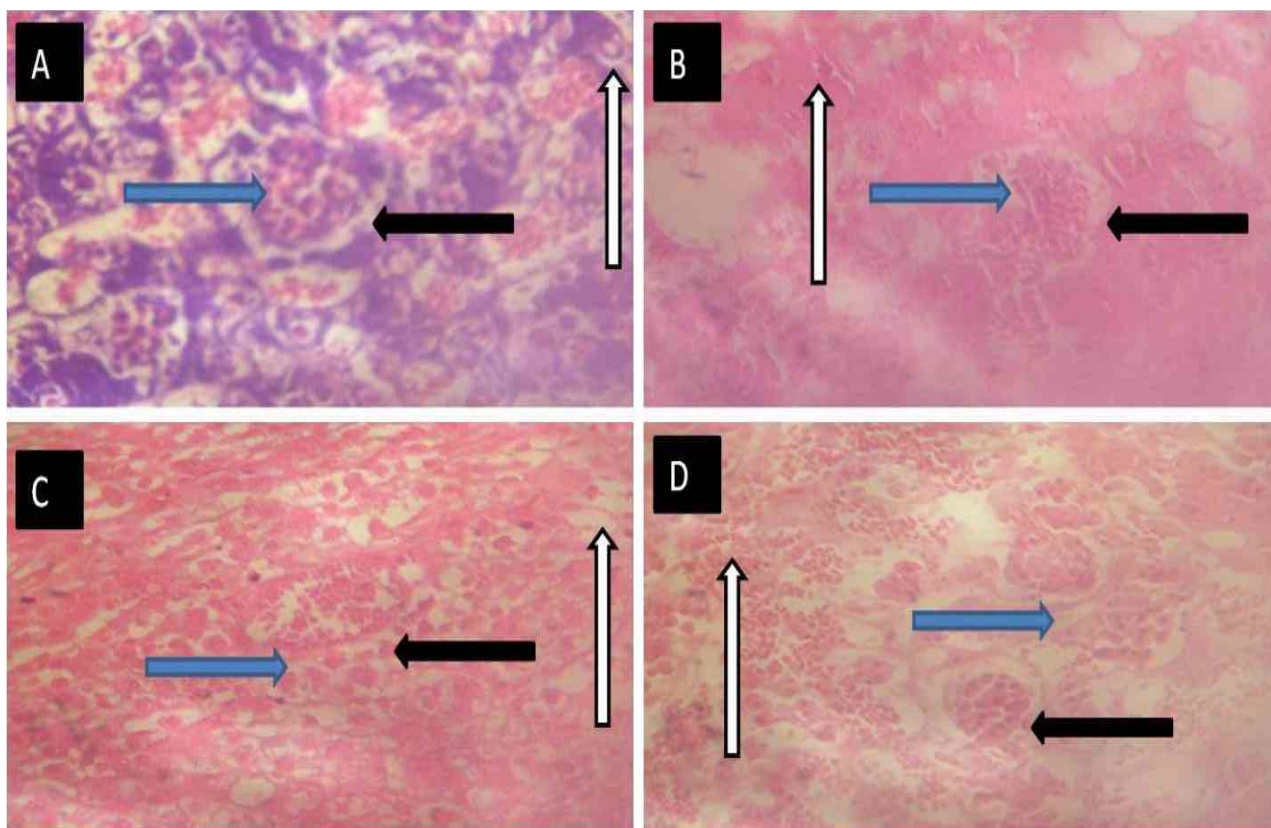
histomorphological features of the Bowman's capsules, glomerulus and blood vessel of the kidney section were well preserved with clear demarcation of the cellular boundaries in plate section stained with H and E as control (plate 1A and 2A). The nucleus of the kidney tissue stained pale red while the cytoplasm stained pale pinkish by 5% extract with potassium alum at pH 4.7 for 20 minutes of time interval. The histomorphological features of the bowman's capsules, glomerulus and blood vessel were well preserved with clear demarcation compared with the control (plate 1B). The nucleus of the kidney tissue stained pale red while the cytoplasm stained pale pinkish by 10% extract with potassium alum at pH 4.0 for 15 minutes of time interval. The histomorphological features of the bowman's capsules, glomerulus and blood vessel were well preserved with fairly demarcation compared with the control (plate 1C). The nucleus of the kidney tissue stained pale red while the cytoplasm stained pale pinkish by 15% extract with potassium alum at pH 3.9 for 10 minutes of time interval. The histomorphological features of the bowman's capsules, glomerulus and blood vessel were well preserved with fairly demarcation compared with the control (plate 1D).



**Plate 1.** A photomicrograph of kidney section stained with haematoxylin and eosin as control (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow). (B) photomicrograph of kidney section stained with 5% alcoholic extract of *saccharum officinarum* mordanted with potassium alum and eosin as test (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow), (C) photomicrograph of kidney section stained with 10% alcoholic extract of *saccharum officinarum* mordanted with potassium alum and eosin as test (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow). (D) photomicrograph of kidney section stained with 15% alcoholic extract of *saccharum officinarum* mordanted with potassium alum and eosin as test (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow).

The nucleus of the kidney tissue stained pale red while the cytoplasm stained pale pinkish by 5% extract with ferric chloride at pH 2.3 for 20 minutes of time interval. The histomorphological features of the bowman's capsules, glomerulus and blood vessel were well preserved with fairly demarcation compared with the control (plate 2B). The nucleus of the kidney tissue stained pale red while the cytoplasm stained pale pinkish by 10% extract with ferric chloride at pH 2.0 for 15 minutes of time interval. The histomorphological features

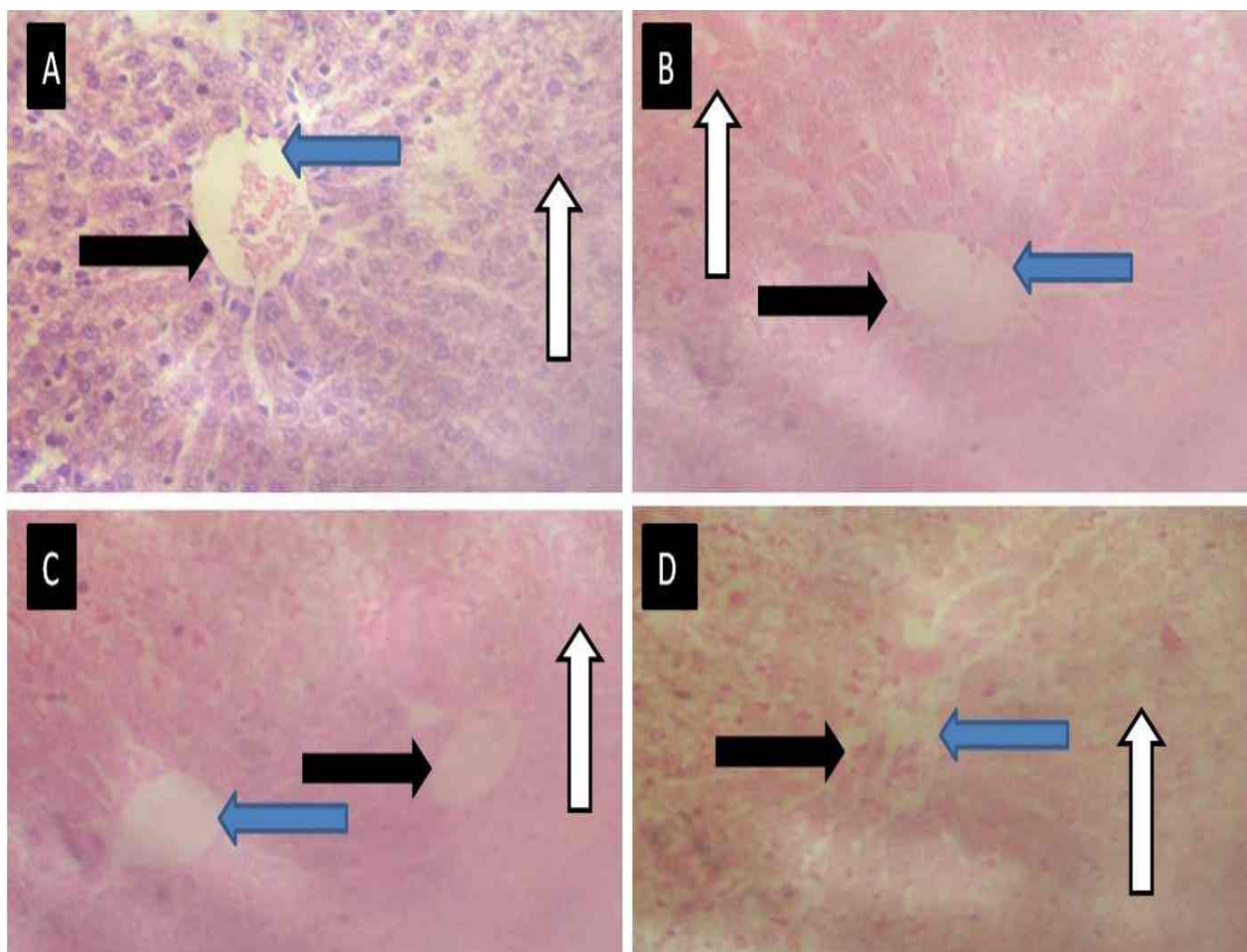
of the bowman's capsules, glomerulus and blood vessel were well preserved with clear demarcation compared with the control (plate 2C). The nucleus of the kidney tissue stained pale red while the cytoplasm stained pale pinkish by 15% extract with ferric chloride at pH 2.1 for 15 minutes of time interval. The histomorphological features of the bowman's capsules, glomerulus and blood vessel were well preserved with fairly demarcation compared with the control (plate 2D).



**Plate 2.** (A) Photomicrograph of kidney section stained with haematoxylin and eosin as control (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow). (B) photomicrograph of kidney section stained with 5% alcoholic extract of *saccharum officinarum* mordanted with ferric chloride and eosin as test (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow), (C) photomicrograph of kidney section stained with 10% alcoholic extract of *saccharum officinarum* mordanted with ferric chloride and eosin as test (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow). (D) photomicrograph of kidney section stained with 15% alcoholic extract of *saccharum officinarum* mordanted with ferric chloride and eosin as test (X400), bowman's capsule (black arrow), glomerulus (blue arrow) and distal convoluted tubules (white arrow).

The nuclear component of liver tissue stained purple while the cytoplasmic components appeared pale pinkish. The histomorphological features of the portal triads, central vein and hepatocytes of the liver section were well preserved with clear demarcation of the cellular boundaries in plate section stained with H&E as control (plate 3A and 4A). The nuclear component of liver tissue stained pale red while the cytoplasmic components appeared pale pinkish by 5% extract with potassium alum at pH 4.7 for 2 minutes of time interval. The histomorphological features of the portal triads, central vein and hepatocytes of the liver section were well preserved with clear demarcation comparable with control (plate 3B). The nuclear

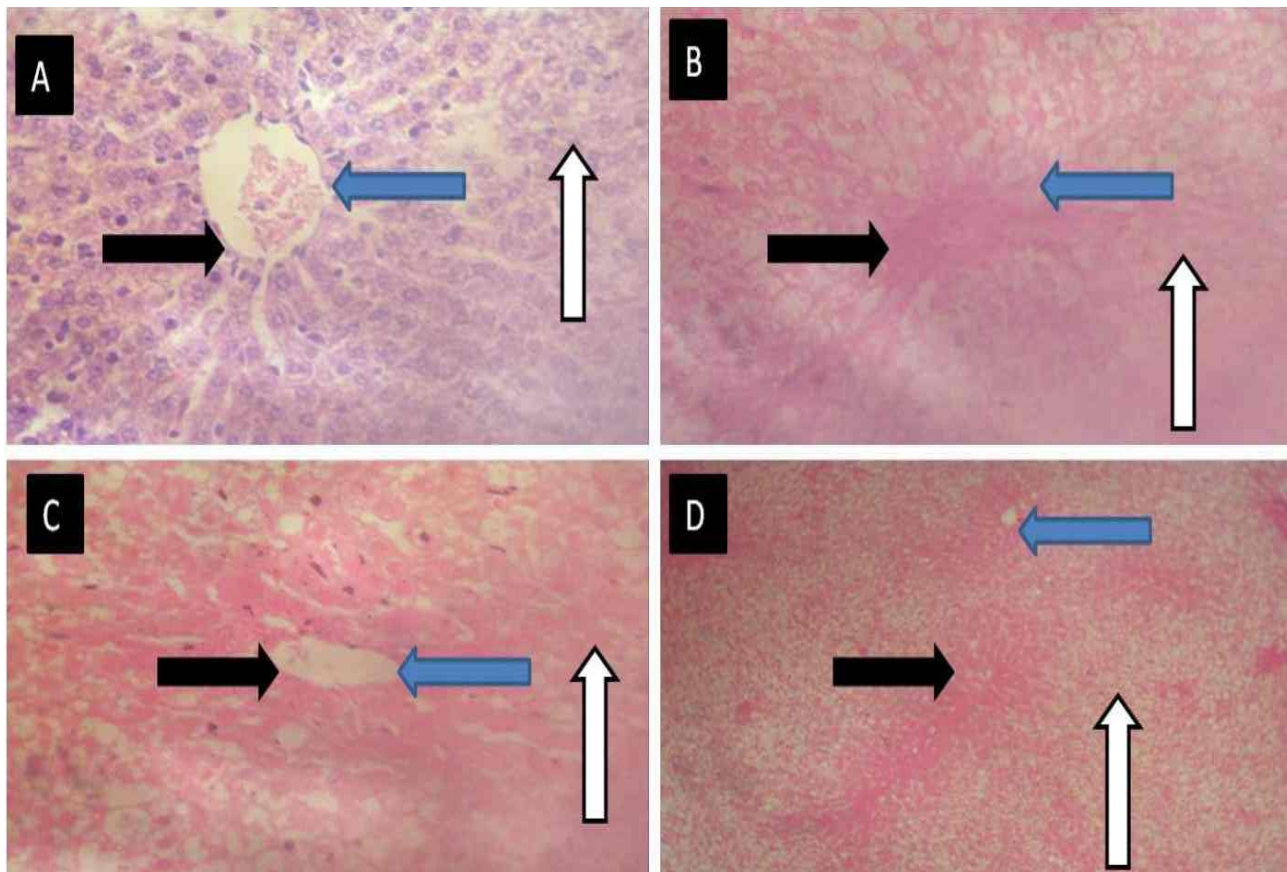
component of liver tissue stained pale red while the cytoplasmic components appeared pale pinkish by 10% extract with potassium alum at pH 4.0 for 15 minutes of time interval. The histomorphological features of the portal triads, central vein and hepatocytes of the liver section were well preserved with fairly demarcation comparable with control (plate 3C). The nuclear component of liver tissue stained pale red while the cytoplasmic components appeared pale pinkish by 15% extract with potassium alum 3.9 at pH 2.3 for 10 minutes of time interval. The histomorphological features of the portal triads, central vein and hepatocytes of the liver section were well preserved with clear demarcation comparable with control (plate 3D).



**Plate 3.** (A) Photomicrograph of liver section stained with haematoxylin and eosin as control (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow). (B) photomicrograph of liver section stained with 5% alcoholic extract of *saccharum officinarum* mordanted with potassium alum and eosin as test (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow). (C) photomicrograph of liver section stained with 10% alcoholic extract of *saccharum officinarum* mordanted with potassium alum and eosin as test (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow). (D) photomicrograph of liver section stained with 5% alcoholic extract of *saccharum officinarum* mordanted with potassium alum and eosin as test (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow).

The nuclear component of liver tissue stained pale red while the cytoplasmic components appeared pale pinkish by 5% extract with ferric chloride at pH 2.3 for 20 minutes of time interval. The histomorphological features of the portal triads, central vein and hepatocytes of the liver section were well preserved with fairly demarcation comparable with control (plate 4B). The nuclear component of liver tissue stained pale red while the cytoplasmic components appeared pale pinkish by 10% extract with ferric chloride at pH 2.0 for 15 minutes of time interval.

The histomorphological features of the portal triads, central vein and hepatocytes of the liver section were well preserved with clear demarcation comparable with control (plate 4C). The nuclear component of liver tissue stained pale red while the cytoplasmic components appeared pale pinkish by 15% extract with ferric chloride at pH 2.1 for 10 minutes of time interval. The histomorphological features of the portal triads, central vein and hepatocytes of the liver section were well preserved with fairly demarcation comparable with control (plate 4D).



**Plate 4.** (A) Photomicrograph of liver section stained with haematoxylin and eosin as control (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow). (B) photomicrograph of liver section stained with 5% alcoholic extract of *saccharum officinarum* mordanted with ferric chloride and eosin as test (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow). (C) photomicrograph of liver section stained with 10% alcoholic extract of *saccharum officinarum* mordanted with ferric chloride and eosin as test (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow). (D) photomicrograph of liver section stained with 5% alcoholic extract of *saccharum officinarum* mordanted with ferric chloride and eosin as test (X400), portal triad (black arrow), central vein (blue arrow) and hepatocyte (white arrow).

### Discussion

The quality of staining in histology depends on the microscopic appearance of cell membrane, nuclear membranes, cytoplasm transparency and extracellular matrix (Benard *et al.*, 2015). *Saccharum officinarum* stain renders a promising colour on the histological sections of some selected organs such liver and kidney. The present study attempted to use the extract of locally available *Saccharum officinarum* mordanted with potassium alum and ferric chloride solution with eosin as counterstain to demonstrate the general architecture of liver and kidney. During staining, dye molecules in stains appear as a certain colour

and attached to a specific site or cellular structure. Combination of stain may be necessary to affect certain tissue demonstration. This goes to show that dye a dye must ionize in solution to produce coloured cation and anions which are capable of uniting with tissue component to form coloured compounds (Mohammed *et al.*, 2020). Five percent 5% alcoholic extract of *saccharum officinarum* bark mordanted with potassium alum stained the liver and kidney tissue section in the shade of pale pinkish and pale red. This staining reaction has a resemblance of haematoxylin staining reaction in a routine haematoxylin and eosin stain. These findings are similar to the report of Benard *et al.*



(2015) and Mohammed *et al.* (2020) where *H. sadariffa* and *Allium cepa* extracts were used to stained sections of skin and liver tissues respectively. The intensity of this staining reaction may be due to the addition of potassium alum. Similarly, 10% and 15% of alcoholic extract of *Saccharum officinarum* bark mordanted with ferric chloride as a mordant stained the tissues pale red with an excellently and well-preserved tissue constituents. These findings vary from the finding of Omoowo *et al.* (2014); Benard *et al.* (2015) where different staining colour patterns were observed. This variation can be attributed to differences in the extraction plant, solvent, concentration, mordant and pH used. While the staining characteristic of the extract have been established in the study, the staining ability of 5% extract mordanted with potassium alum, 10% and 15% mordanted with ferric chloride were found to be remarkably impacted on the tissue with potassium alum as mordant showing the best staining ability and may be substituted for haematoxylin as a primary stain in haematoxylin and eosin staining technique.

### Conclusion

The staining characteristics of the tissue were well demonstrated, to the hematoxylin and eosin-stained section. The use of alcoholic extract of *Saccharum officinarum* bark as a primary stain showed that the extract was a good primary stain as it stained. It may therefore be concluded that 5% alcoholic extract of *Saccharum officinarum* bark, mordanted with potassium alum and 10% and 15% mordanted with ferric chloride is a promising histological stain.

### Recommendation

Based on the findings, it is recommended that the extract of *Saccharum officinarum* bark should be tried as primary stain with eosin as a counter stain. Further studies should be conducted to harness the potential of *Sacharum officinarum* extract as a stain in histopathology laboratory.

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