



**Original article**

## **Kola nut (*cola acuminata*) extract as a substitute to histological tissue stain eosin**

**S.A. Shehu<sup>a</sup>, M.L. Sonfada<sup>a</sup>, A. Danmaigoro<sup>a</sup>, A.A. Umar<sup>a</sup>, S.A. Hena<sup>a</sup>, I.M. Wiam<sup>b</sup>**

<sup>a</sup>Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto

<sup>b</sup>Department of Veterinary Anatomy, University of Maiduguri, Maiduguri

\*Corresponding author; Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto

### ARTICLE INFO

### ABSTRACT

*Article history:*

Received 14 July 2012

Accepted 19 July 2012

Available online 28 August 2012

*Keywords:*

Kolanut extract

Substitute

Histological tissue

Eosin

The application of natural dyes for staining of various biological tissues from an alternative source will decrease the expense for purchasing the synthetic dye and reduce their effects on human and environment. Therefore, the objective of this study was to investigate the extraction of natural dye from Cola nut (*Cola acuminata*) using various solvents and its staining property on the rat tissues. The cola nut was pulvized using pestle and mortar, 5gram was used to make 5% of aqueous extract. The rat tissues were processed for paraffin embedding technique and sectioned at 5  $\mu$ m thicknesses. The sections were stained with haematoxylin and the extracts as secondary stain. The results showed that the natural extract from *Cola acuminata* stained the cytoplasm of various tissues with yellowish-brown colouration. This finding suggests that *Cola acuminata* can be used as an alternative dye for histological staining.

© 2012 Sjournals. All rights reserved.

### **1. Introduction**

In histology, there are two types of dyes, Natural dyes obtained from natural sources and Synthetic dyes produced through chemical reactions (Avwioro, *et al.*, 2005; Carleton, *et al.*, 1976). The synthetic dyes are very efficient, but they are hazardous to human and animal health (Bhuyan, *et al.*, 2004). Thus, the searching of new natural dyes for histological staining that are eco-friendly and biodegradable necessary.

Cola is a genus of about 125 species of trees, native to the tropical rainforest of Africa (Benjamin, 1991). There are several kinds of cola seeds derived from different species, but *Cola vera* and *Cola acuminata* are mostly used and preferred for medicinal purposes (Ayensu, 1978). The seeds are chewed to curb hunger, allay thirst, and enable people to work hard in hot conditions (Ayensu, 1978). Kola Nut seeds are considered a symbol of hospitality and used in many social ceremonies such as marriage, birth and funerals. It was observed that those engage in constant kola nut chewing have their gums and teeth stained (Cheek, 2002). However, staining with *Cola acuminata* has not been investigated. Recent move to make use of local natural resources to minimize total dependence on foreign, imported products in order to improve economic values of our local material has been made. Positive result has been documented from the use of *Lawsiana inermis* and *Hibiscus sabdariffa* to stain histological tissue (Wiam, et al., 2006). Synthetic dyes are increasingly becoming expensive to our laboratories where funding is limited. It is for these reasons that an alternative natural dye which is cheaper and bio-friendly is been sort as potential natural histological dye.

## 2. Material and methods

The plant sample *C. acuminata* was purchased from Sokoto Central Market and was identified at the herbarium of Department of Biological sciences, Faculty of Science, Usmanu Danfodiyo University Sokoto. The Kola nut seed was mashed using perforated empty tin can and pulverized using Mortar and pestle. Five (5) grams of the powder was weighed using Mettler's balance and mixed in 100ml of distilled water and the extract was kept for 24 hours, which was later sieved with cotton wool followed by a folder filter paper placed in a glass funnel and Conical flask. The filtrate was labeled 5% aqueous extract of the *C. acuminata*.

### 2.1. pH Determination

Digital BMF/pH meter (England) was used for determination of pH value of dye.

### 2.2. Histological investigation

One apparently healthy rat was sacrificed and organs (Intestine, Liver, Kidney, Cardiac muscle and Lungs) were removed and fixed in 10% buffered formalin for 72 hours to fix properly and processed for paraffin embedding technique (Bancroft, 2002) and sectioned at 5  $\mu$ m thickness. The technique for normal Haematoxylin and Eosin (H & E) was followed.

The tissue slides were grouped and are deep in the two (2) different prepared solutions which are as follows: A: 5% aqueous extract of *C. acuminata*, B: *Eosin*, which serve as the control group and lastly the mounted tissues were covered with cover slip using Canada balsam mountant.

The tissues were placed under a light microscope with x400 magnification and image of the tissue were capture using a motic camera (Moticam 1000, 1.3 mega Pixel) and the photomicrograph were transferred to the computer. The quality and stability of *C. acuminata* extract were examined for the intensity of nucleus and cytoplasm staining.

## 3. Results and discussion

The pH of the aqueous extract of the *Cola acuminata* was 5.93 making it acidic. Giving by the theory of neutralization reaction, acidic dyes stain cytoplasm while the basic dye, stain the neuleus, the pH of the extract implies that the substance is an acidic, thus it choice of cytoplasmic stain. As shown on plates i – x, the aqueous extract has demonstrate the property of neutralization reaction.

In this research, Haematoxylin and aqueous *C. auminata* extracts stained the nucleus and cytoplasm of tissues with bluish and yellowish coloration respectively. The intensity of their staining was not significantly different from those stained with haematoxylin and eosin as shown in plate i - x

The results suggest that the dye from *C. acuminata* can be extracted by either acidic or neutral solvents, the acidity and alkalinity of media do not affects the staining quality of *C. acuminata* extract, like in the *Peterocarpus osun* dye as reported by Avwioro et al.,(2005).

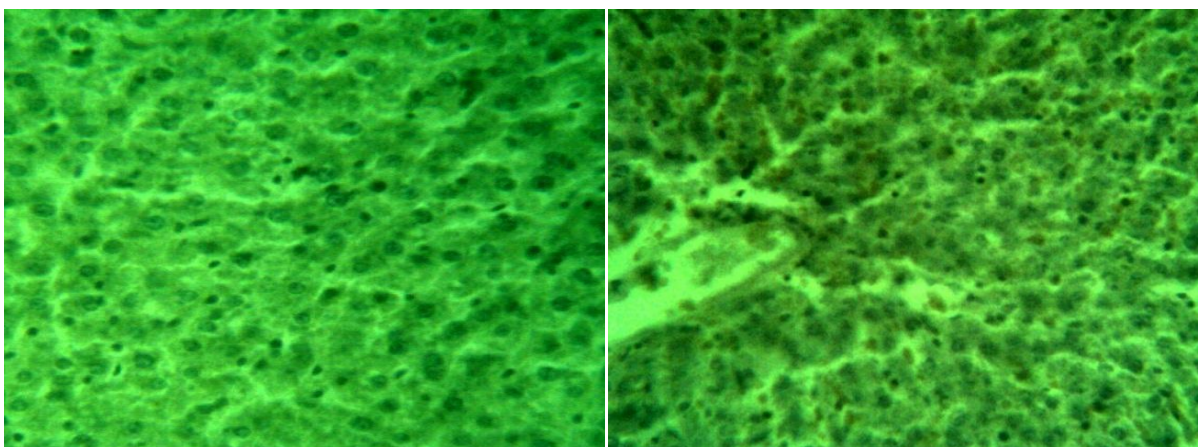
Besides the pH of the extract, mordant has been reported to affect some stains (Avwioro et al., 2005). In some studies of natural extract, Black plum (*Syzygium cumini*) mordant was used (Papawee, et al., 2011) However, in

case of this study, no mordant was used and as such no significant effect on the staining qualities of *C. acuminata* extract was noticed. This is unlike most dyes used in histochemistry such as hematoxylin, which is first oxidized to hematein and mordant is added before it can be used as a stain for tissues as reported by Bancroft, (2002) and Avwioro *et al.*, (2005).

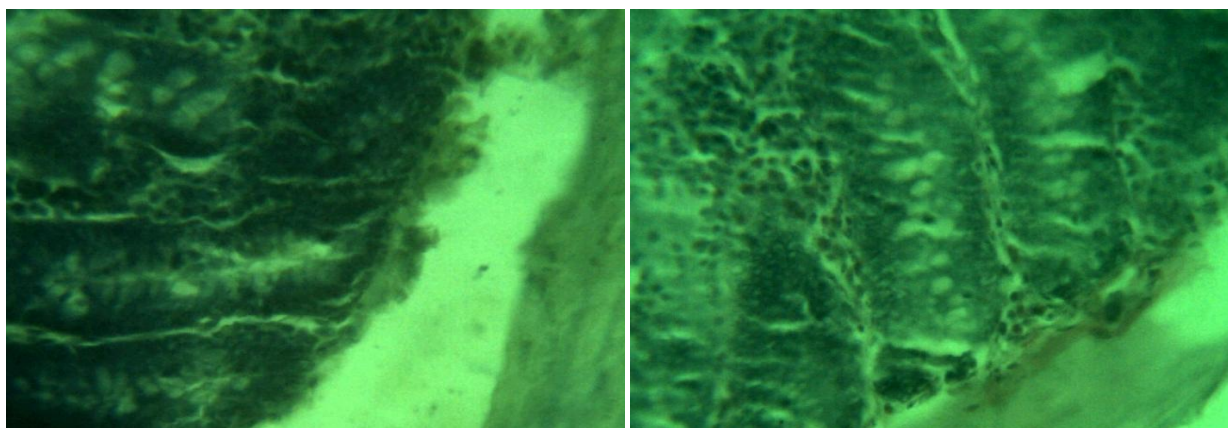
The ability of a dye to stain specific tissue structures is determined by certain factors such as the electrostatic attractions. Acidic structures (e.g. nucleus) are stained by basic dyes (e.g. hematoxylin) while basic structures (e.g. cytoplasm) are stained with acidic dyes (e.g. eosin) (Avwioro *et al.*, 2005; Avwioro, O.G. 2002). In this study, the natural dye from *C. acuminata* stained the cytoplasm of the tissues processed. The chemical components of *C. acuminata* dyes may probably be the Hydro ( $H^+$ ) polar molecule in respect to the pH that is found to be 5.93. This study was in agreement with the studies conducted by Avwioro *et al.*, (2005) which showed that the red dye stuffs obtained from *Pterocarpus osun* species were used in staining tissue section for histopathological diagnosis of diseases. However, the nut of *C. acuminata* is reported to contain vitamin C, Gallic acid, tannins, anthocyanins cyaniding glucoside, petunidin, malvidin and other components that act as antioxidant property for medicine and cosmetic.

#### 4. Conclusion

*Cola acuminata* extracts could be used to stain cytoplasm of body tissue with a yellowish-brown coloration, while serving as an alternative natural dye for histological staining.

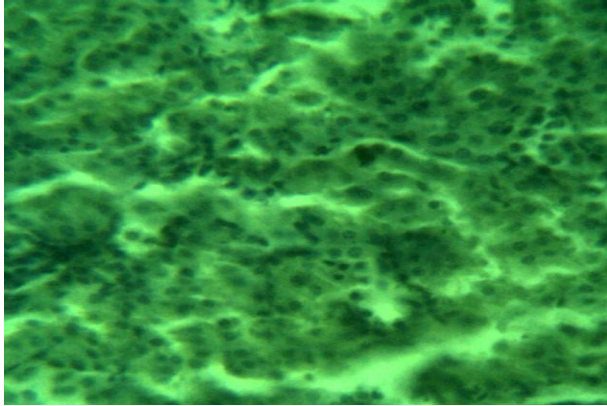


**Plate i.** Rat liver (Haematoxylin & Kola extract x400). **Plate ii.** Rat liver (Haematoxylin & Eosin x400).

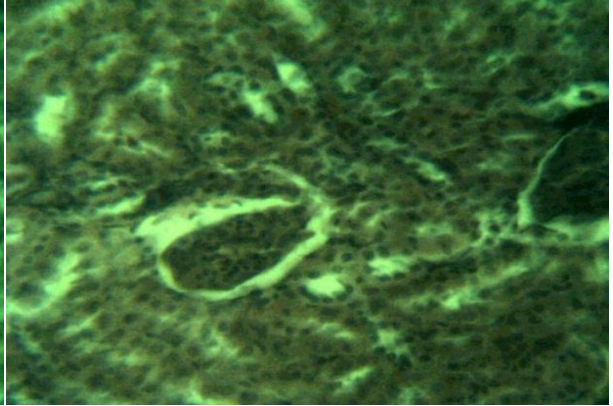


**Plate iii.** Rat Intestine (Haematoxylin & Kola extract x400). **Plate iv.** Rat intestine (Haematoxylin & Eosin x400).

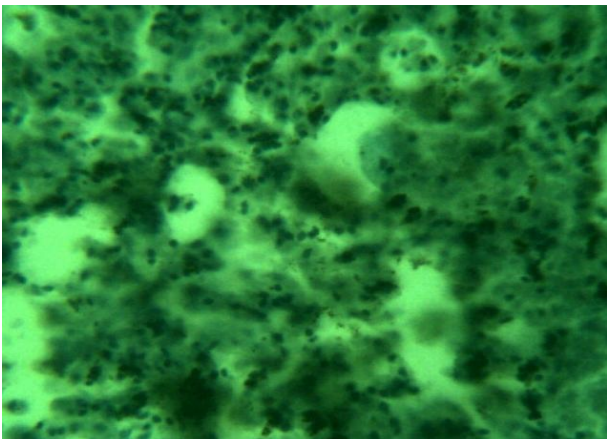




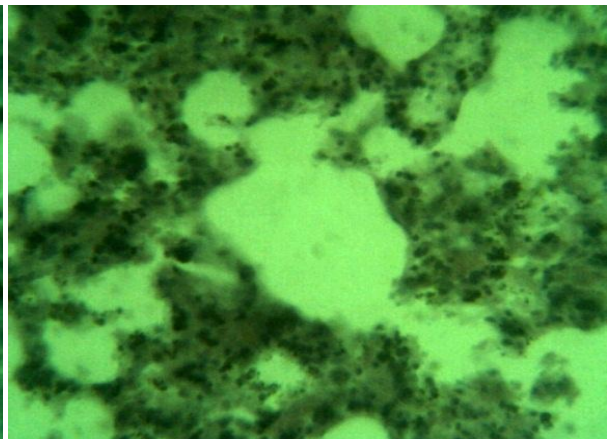
**Plate v.** Rat Kidney (Haematoxylin & Kola extract x400).



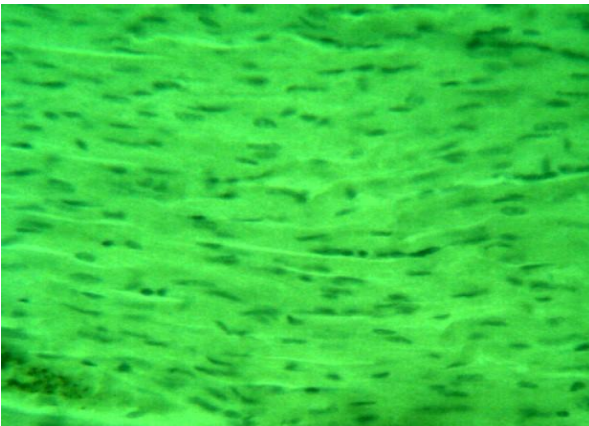
**Plate vi.** Rat Kidney (Haematoxylin & Eosin x400).



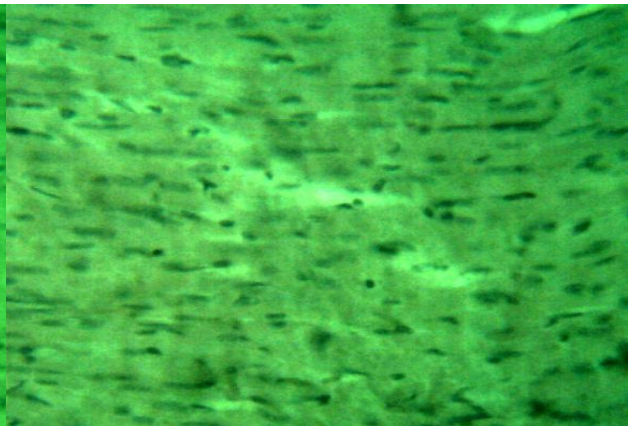
**Plate vii.** Rat Lung (Haematoxylin & Kola extract x400).



**Plate viii.** Rat Lung (Haematoxylin & Eosin x400).



**Plate ix.** Rat Cardiac Muscle (Haematoxylin & Kola extract x400).



**Plate x.** Rat Cardiac Muscle (Haematoxylin & Eosin x400).

#### Acknowledgements

This study was supported by the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

#### References

- Avwioro, O.G., Aloamaka, P.C., Ojiana, N.U., Oduola, T. Ekpo, E.O., 2005. Extracts of *Pterocarpus osun* as a histological stain for collagen fibres, African Journal of Biotechnology. 4 (5), 460-462.
- Avwioro, O.G., 2002. Histochemistry and tissue pathology, 1st ed., Claverianun press, Nigeria,
- Ayensu, E. S., 1978. Medicinal plants of West Africa, Reference Publication International, Michigan.
- Baker, F.J., Silverton, R.E., Palister, C.J., 2001. Introduction to Medical Laboratory, 7th edn., Bounty Press Limited, Ibadan, Nigeria, pp. 258-261.
- Bancroft, M. G., 2002. Theory and Practice of Histological Techniques, Churchill Livingstone, London.
- Benjamin, L.T Jri., Rogers, A.M., Roenbaum, A., 1991. Coca-cola, caffeine and mental deficecny; Harry Hallingworth and the Chattanooga trial of 1911; Journal of History behaviour science 27(1) 42 - 55.
- Bhuyan, R., Saikia, C.N. (2004). Isolation of colour components from native dye-bearing plants in Northeastern India, Am. J. Pathol. 164 (5), 873-77.
- Cheek, M., 2002. Three new species of Cola (Sterculiaceae) from western Cameroon. Kew Bulletin (Royal Botanic Gardens, Kew) 57 (2): 403
- Drury, R.A.B. and Wallington. E.A., 1976. Carletons Histological Techniques, 4th edition, Oxford University Press, London, UK.
- Papawee, S., Suppaluk, R., Natthawut, T., 2011. Extraction of Natural Histological dye from Black Plum fruit (*Syzygium cumini*) Journal of the Microscopy Society of Thailand 4(1), 13 – 15.
- Wiam, I.M., Sonfada, M.L., Oke, B.O., Kwari, H.D and Onyeyili, P.A., 2006. *Lawsonia inermis* and *Hibiscus sabdariffa*: Possible Histological stains. Tropical Vet. 22: (1 & 2) 1 -5.