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Camwood (*Baphia nitida*) alcoholic extract: A suitable counter stain for haematoxylin in the demonstration of liver and kidney histomorphology

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The counter staining ability of Camwood (*Baphia nitida*) alcoholic extract and its electrostatic compatibility with haematoxylin was determined. One gram of locally sourced red paste was subjected to simple extraction in 100 ml of 70 and 95% alcohol, respectively. Primary staining of buffered formalin fixed, paraffin embedded liver and kidney tissues was done with haematoxylin followed by counter staining with extract solutions at different experimental times of 1, 5, 30 and 60 min respectively. Parallel staining was done with H&E as control. Results show impressive nuclei-cytoplasm contrast at 60 min. It was therefore concluded that *B. nitida* is a suitable counter stain for haematoxylin stains especially on liver and kidney tissues and may be a potential alternative to eosin in the demonstration of tissue morphology.

Key words: Camwood, haematoxylin, natural dye, liver, kidney.

INTRODUCTION

Baphia nitida Lodd belongs to the Fabaceae family commonly known as Camwood. It is very abundant in under wood of the African dense forests. The plant is also known as Camwood, barwood or African sandlewood (Oli et al., 2017). Camwood (*B. nitida*) is of the genus *Pterocarpus osun* which belongs to the family *Papilonaceae.* It is also known as African sandalwood; is a shrubby, leguminous, hard wooded tree which is found generally in the tropical areas of the globe particularly Africa and Asia where they are used for the management of a wide range of ailment (Green, 1995).

B. nitida lodd (Camwood), a leguminous, shrubby, hard-wooded tree belonging to the sub-order Cesalpinieae and family Fabaceae, is a widespread forest plant distributed around the globe and is common within the coastal region of Africa (Chibuisi et al., 2015). It is used to add colour to locally made palm kernel oil known as 'Mmanuaki'. In Europe, redwood is used to add colour

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and flavour to sea foods, sauces meat products, snacks, food and alcoholic drinks (Thadens and Verstrygne, 1989). The principal pigments are santalin A&B and Santarubin which are insoluble in water but soluble in organic acid and alkali (Green, 1995).

Leaves and bark from this plant are considered hemostatic and anti-inflammatory and are used for curing sores and wounds, while the dye from the bark is formed into red body cosmetics. Honey-hunters rub their body with the dye to prevent bee-stings. It is also made into ointments which are applied against stiff and swollen joints, sprains and rheumatism. The plant materials are increasingly being used as a major ingredient in the production of local cosmetic and skincare products (Chibuisi et al., 2015).

The powdered stem prevents infection of the freshly severed umbilical cord. The dry leaf is also an ingredient of traditional black soap, while the heartwood, bark and roots are pounded into a paste and used as skin cosmetic. Wood from this burgundy coloured tree wood is extremely hard, heavy and durable and is traditionally used to make drum sticks, mortars and pestles, knife handles and similar articles. It is also used to make ornamental fencing in areas of South West Nigeria. In the Yoruba language, it is known as *osun* (Green, 1995) traditionally.

Phytochemical screening of Camwood reveals that it contains flavonoids and a trace of alkaloids (Agwa et al., 2012). Other constituents of the plant include saponins, phlobatannins, terpenes and cardiac glycosides (Joseph et al., 2013). Its possession of significant antimicrobial activity and hence possible use as a remedy for pathogenic infections has been well documented (Agwa et al., 2012). The plant has also been found to protect against toluene induced toxicity in rats hence safe for use in skin care products and cosmetics (Chibuisi et al., 2015). Toxicological assessment of Camwood alcoholic extract has shown that the plant has no damaging effect on the liver and kidney of albino rats. It has also been known to have anti-oxidative effect (Akande et al., 2011).

There are two types of dyes; natural dyes obtained from natural sources and synthetic dyes produced through chemical reaction (Carleton et al., 1976; Avwioro et al., 2005; Araya and Nattakarn, 2014). Most histological stains in current use are of synthetic origin, however, natural dyes are still promising to be cheaper potential sources (Mattuk, 1998). Any development of new histological stain is justified if the new stain is cheaper, available, harmless and easier in application (Penney et al., 2002; Muhammed et al., 2016). Therefore, the use of naturally occurring dyes from plants which is less expensive is being viewed as an alternative to synthetic dyes (Araya and Nattakarn, 2014).

Camwood (*B. nitida*) will be of great contribution to the exploitation of natural dyes and their applications. This research wants to contribute to the use of natural dyes from Camwood as counter stain to haematoxylin in the histological staining of liver and kidney.

MATERIALS AND METHODS

Sample collection

10% neutral buffered formalin (NBF) fixed, paraffin embedded tissue blocks of liver and kidney tissues were retrieved from the archive of the Pathology Department, University of Ilorin Teaching Hospital.

Sourcing of Camwood powder

Camwood powder was purchased at a local market (Oja Titun) in Ilorin, Kwara State and identified at the Department of Plant Science, University of Ilorin. The red powder was marched with the herbarium archive specie and given identification number UILH/002/1157.

Extraction with alcohol

1 g of the Camwood powder was weighed with a digital weighing balance (Ohaus) and dissolved in 100 ml of 70% alcohol, filtered with a Whatman filter paper, labeled solution A and stored at room temperature. Subsequently, another 1 g of the Camwood powder was weighed in with a digital weighing balance (Ohaus) and dissolved in 100 ml of 95% alcohol, filtered with a Whatman filter paper, labeled solution B and stored at room temperature.

Slide preparation and staining

Eight (8) serial sections labeled A-J were made from each block of liver and kidney using a LEICA rotary microtome at 4 microns and stained with Harris haematoxylin. A-D was counterstained with 70% alcoholic Camwood for 1, 5, 30 min and 60 min, respectively. F-H was counterstained with 95% alcoholic Camwood for 1, 5, 30 and 60 min, respectively. Parallel controls were stained with H&E. Sections were dewaxed in xylene and hydrated through 100, 90, 70 and 50% alcohol to water and subsequently stained in Harris haematoxylin for 15 min, washed in running tap for 2 min, differentiated in 1% acid alcohol, washed in running tap water for 10 min, counter stained with alcoholic Camwood solutions and mounted in DPX. For the H&E procedure, sections were dewaxed in xylene and hydrated through 100, 90, 70 and 50% alcohol to water; and subsequently stained in Harris haematoxylin for 15 min, washed in running tap for 2 min, differentiated in 1% acid alcohol, washed in running tap water for 10 min, counter stained in 1% alcoholic eosin for 30 s and mounted in DPX.

Slide assessment

Stained slides were blind assessed independently by three histopathology experts for cellular appearance, cytoplasmic-nuclei background staining and differential staining contrast.

RESULTS AND DISCUSSION

Stains are substances, which colour tissues in order to aid optical differentiation of tissue components (Avwioro, 2014). Cellular structures are selectively stained by various natural and synthetic dyes. Some require

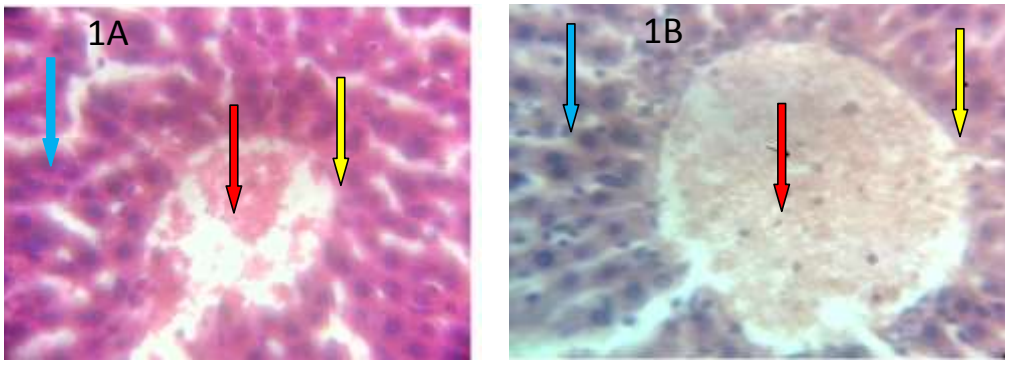

Figure 1. Photomicrograph 1A: showing liver with nucleus (blue arrow), rbc (red arrow) and cytoplasm (yellow arrow){H&E Control}; 1B: Showing Liver with nucleus (blue arrow), rbc (red arrow) and cytoplasm (yellow arrow) (Haematoxylin-Camwood). Mag. X 200.

combination of stains to demonstrate the presence of some of these tissue structures (Avwioro et al., 2005).

Few works exist on the histopathological application of Camwood extract. Apart from the work of Fasan (1972), who experimented on the histological application of *B. nitida* extract on dentine tissue, much work has not been done until years later when other researchers started exploring the diagnostic use of this natural plant. Avwioro and colleagues (2005) experimented on the staining ability of *P. osun* extract on tissue sections. They used the alcoholic and acidic extracts to stain different tissues and reported that collagen fibres, red blood cells and muscles were stained in shades of reddish brown. They concluded that *P. osun* extract is a promising histopathological stain that can serve as a useful stain for histopathological diagnosis of diseases. Later on, extracts of plants from kola nut were used as histological counter stain to haematoxylin with a yellowish-brown colour (Shehu et al., 2012). They recommended it as an alternative histological stain for its eco-friendly characteristics.

Earlier researchers did not concentrate on the eosinophilic staining ability of Camwood powder (*B. nitida*) and its electrostatic compatibility with haematoxylin as counter stain. Prospects for natural dyes as possible histological stains had made more researchers to experiment with other local dyes like *Sorghum bicolor*, *Hibiscus sabdariffa* and *Hibiscus rosa-sinensis* extract as counter stain to haematoxylin (Omoowo et al., 2014; Ibnouf et al., 2014; Raheem et al., 2015; Sudheendra et al., 2016). In this study, Camwood was investigated as a prospective counter stain to haematoxylin. Nuclei stained purple-blue while cytoplasm stained pinkish red. Best eosinophilic staining was achieved at 60 min comparable with standard H%E. Low intensity of staining was observed in the alcoholic extract of the Camwood counter stain at short staining time while high intensity of staining was observed at long staining time. Liver hepatocytes, nucleus, renal corpuscles, bowman capsules and cytoplasm were differentially stained with good contrast. In our study, the dye was applied as a histological counter stain on liver and kidney. This is to contribute to knowledge on the morphological appearance of haematoxylin-Camwood stained tissue with the advantage of assessing the nuclei-cytoplasm integrity and clarity of cellular components. This is in contrast to the earlier work by Avwioro et al. (2005) who applied extract of the dye on skin with no particular interest in nuclei-cytoplasmic interactions as it concerns simultaneous application of haematoxylin. *B. nitida* in this study showed satisfactory interaction with haematoxylin and good electrostatic compatibility perhaps due to the positive and negative ionic charges of haematoxylin and Camwood respectively. Different methods of extraction of dye have been used by researchers over time. This ranges from ordinary water to hot water, acid extraction and alcoholic extraction (Araya and Nattakarn, 2014; Shehu et al., 2012; Oli et al., 2017). In this study, simple alcoholic extracts (70 and 95%) were used. The difference in the concentration of alcohol used for extraction had no significant effect on the staining ability of the Camwood extract as counter stain to haematoxylin on liver and kidney tissues. This is to say that *B. nitida* dissolves well in both 70 and 95% alcohol. Interestingly, simple alcoholic extracts of natural dyes had shown promising outcomes in previous studies (Omoowo et al., 2014; Muhammed et al., 2016; Sudheendra et al., 2016).

The hepatic nuclei, cytoplasm and red blood cells of the liver were well stained by the haematoxylin-Camwood method comparable with that of standard haematoxylin and eosin staining technique (Figure 1).

The renal corpuscles, glomerulus, basement membrane and cytoplasm of collecting ducts of the kidney were also well stained with good contrast by the haematoxylin-Camwood method comparable with the standard H&E (Figure 2).

The impressive contrast observed in this study by the haematoxylin-Camwood method showed good

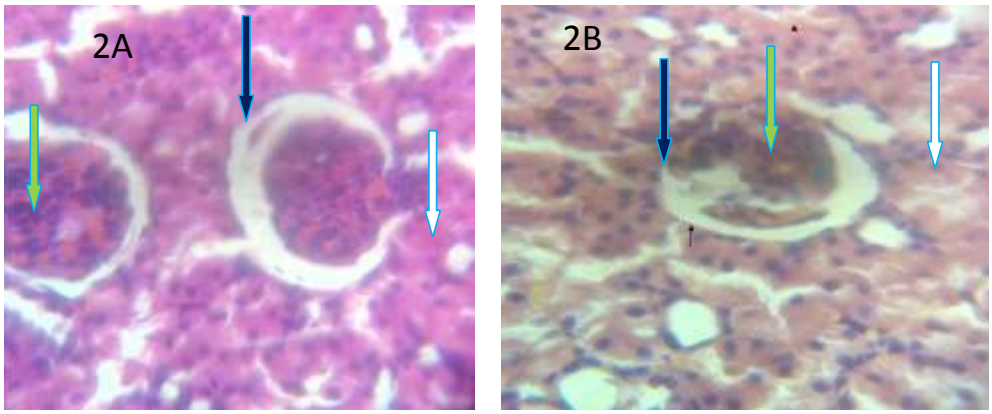

Figure 2. Photomicrograph 2A: showing kidney with bowman's capsule (green arrow), basement membrane (black arrow) and cytoplasm (white arrow) (H&E Control); 2B: showing renal corpuscles (green arrow), basement (green arrow) and cytoplasm (white arrow) by haematoxylin-Camwood method. Mag. X 200.

electrostatic compatibility. Avwioro et al. (2005) reported that basic tissue elements such as cytoplasm will have affinity for an acid stain. The affinity of the dye for the cytoplasm in this work has confirmed that Camwood (*B. nitida*) is an acid dye and it is a promising histological stain which can combine electrostatically with haematoxylin. It is also a promising stain for histopathological diagnoses of diseases.

Conclusion

B. nitida (Camwood) has been revealed to be a suitable counterstain for haematoxylin in the demonstration of liver and kidney histomorphology and could be an alternative to eosin. It is therefore recommended as a good counter stain for biological tissues. The long counter staining time of one hour could be a major limitation for routine application.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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