

## ALTERNATIVE MOUNTING MEDIA IN WOOD HISTOLOGY

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### **Abstract:**

*Wood varnish and clear nail varnish were investigated for their suitability as mountants in this study as most of the mountants used in wood histology are costly and could be toxic. Wood sections of about 20 microns from *Nauclea diderichii* were used; clear varnishes from Soligard Hardglaze Wood Lacquer (WL) and clear nail varnish (CNV) were used as mountants and compared with Canadian balsam which is one of the conventional mountants in wood histology. The results showed that WL and CNV gave good images as also got from Canadian balsam without any distortion (whether shrinkage or other dimensional changes) or any notable difference. Sections prepared in WL varnish were darker as they seemed to have retained more safranin stain. Setting period for WL and CNV was less than 5 hours as opposed to over 24 to 48 hours for Canadian balsam, while that of CNV was the shortest: it set within one hour. Moreover, specimens could be transferred from alcohol and covered directly with CNV during mounting procedures without clearing. Wood cells were observed under a Zeiss light microscope at 80x. Photographs illustrating the micrographs of sections prepared from the two varnishes and Canadian balsam were obtained using a digital camera on the Zeiss light microscope.*

**Key words:** CNV; histology; mountants; CNV; WL.

### **INTRODUCTION**

A mountant protects the specimen; while it also bonds the specimen, cover slip and the slide together, it enhances the rendition of the specimen (Ravikumar *et al.* 2014). Mounting procedure in histology is the last stage in the series that ends with a permanent preparation well after some operations like fixing, embedding, sectioning, staining, dehydrating and clearing (Dioni 2002) have been achieved. The choice of mountant (mounting medium) depends on the type of specimen to be observed and on the type of microscopic technique to be used. Though mounting media are usually resins which are either natural or synthetic, they may be liquid, gum or resinous, soluble in water, alcohol or other solvents (Brown 1997) and some may need to be sealed from the external atmosphere by non-soluble ringing media. Generally, mountants can be classified into two major categories: the resinous mountants which are hydrophobic, adhesive and non aqueous, e.g. Canadian balsam, and the aqueous mountants which are hydrophilic and non adhesive, e.g., glycerine, gum arabic (Culling *et al.* 1985). Canada balsam is a natural resin which happens to be the most well known mountant prepared from the blisters in the bark of the balsamea (*Abies balsamea*) fir tree (Ochei and Kolhatkar 2008), while synthetic resins (e.g. DPX) are made by dissolving a polystyrene in an aromatic hydrocarbon solvent into which a plasticizer is added, though, the actual formulation or preparation of these synthetic resins is kept as a trade secret. Nail polish (or lacquer, or enamel) is a type of synthetic resin designed to protect and beautify nails, it is a highly specialised and flexible lacquer that does not crack and flake with natural nail movement (Toedt *et al.* 2005), though the formulation has been revised repeatedly to enhance its decorative effects and to suppress cracking or flaking. Nail polish consists of a solution of an organic polymer and several other components, depending on the brand. The three major ingredients are organic solvents, resins (thickeners or hardening agents), and colour pigments; a basic clear polish could be made from nitrocellulose (cellulose nitrate) dissolved in butyl acetate or ethyl acetate. Modern nail polish is sold in liquid form in small bottles and is applied with a tiny brush, and hardens to form a shiny coating on the fingernail that is both water and chip resistant. Several studies had been done on nail polish (Microbe 2016; Shahrazad 2005), it can be used to seal the sides of the cover slip when using aqueous mounting media: in microbiological laboratories the fingernail polish material predominantly has been used in sealing the edges of cover slip with water soluble mounting media (Pickard *et al.* 2002; Sturbaum *et al.* 2001; Sturbaum *et al.*

2001), and the slide dries in no more than an hour. Colourless clear fingernail polish is a very common cosmetic material, thus it is easy to find and buy. While the adhesion strength of some wood varnishes had also been investigated (Ozdemir *et al.* 2015), they are generally used to show the beauty, colour and pattern resulting from wood along a film layer (Kaygin and Akgun 2008). A wood varnish is a clear, hard solution of low viscosity that is principally applied (often times by spraying) to wood to give it a glossy finish while forming a protective film around it. Coating wood surface with different types of finishes will not only improve its appearance but also extend its service life. The term varnish refers to the finished appearance of wood product. A distinction between spirit-drying (and generally removable) lacquers and chemical-cure varnishes (generally thermosets containing drying oils) is common, but varnish is a broad term historically and the distinction is not strict. In any case, whether natural or synthetic, mounting media should ideally have a refractive index (RI) close as possible to that of the fixed tissue (approximately 1.53) as this will render the tissue transparent with only the stained tissue elements visible; for instance, xylene which is used as a clearant has a RI very close to the fixed tissue, thereby inducing a certain amount of transparency (Renshaw 2007). Of course with Canada balsam there is historic experience especially in the area of storage ability, but in spite of the attributes possessed by the conventional mountants (e.g. Canadian balsam), it is noteworthy that most of the mountants used in wood histology are costly (Dioni 2002); a bottle of Canadian balsam (100ml) could cost around N3, 800 (about 10 USD), but a bottle of some other types of resins cost much far less; a bottle of nail polish which usually comes in 12 to 20 ml is purchased at the rate of N300 (about 2USD), and the same volume of a wood varnish is cheaper. Moreover, other disadvantage of Canadian balsam is that becomes yellow with age (Ravikumar *et al.* 2014). Therefore, it is necessary to try other alternatives which possess all the afore-mentioned qualities and are still affordable. Hence, this study is intended to investigate clear varnishes such as clear nail varnish and clear wood varnish on their suitability as mountants with a view to including them as suitable mounting media in wood histology.

#### **MATERIALS AND METHOD**

Wood samples from *Nauclea diderichii* were used for this study. The choice of wood species was informed on the basis of its density (medium to high density) and its distinct microscopic features. Wood sections of about 20µm thick were prepared in three planes namely cross sectional, tangential and radial sections using a Reichert sliding microtome. Sections to be mounted in clear nail varnish (CNV) were washed with distilled water and covered with safranin for two minutes dehydration was done by passing the wood sections through a series of bath of increasing concentrations of ethanol. The specimens were later mounted directly without clearing using a clear nail polish. Sections to be mount in clear wood varnish were cleared in a vegetable oil (Adeniyi *et al.* 2016) after passing through staining, washing and dehydration processes. Mounting was done using Soligard Hardglaze Wood Lacquer polyurethane clear varnish (WL). Wood cells were observed under a Zeiss light microscope at 80x. Photographs illustrating the micrographs of sections prepared from nail polish, wood varnish and Canadian balsam were obtained using a digital camera on the Zeiss light microscope. Nomenclature and cell sizes were determined following microscopic terminology for hardwood identification (IAWA Committee 1989).

\*CNV=clear nail varnish, WL= wood lacquer

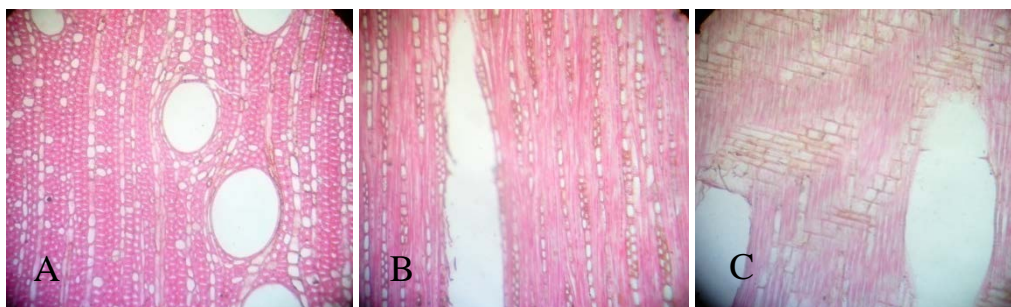
#### **RESULTS AND DISCUSSION**

Sections prepared in clear nail varnish (CNV) and wood varnish revealed all the wood features (Figs. 2-4) clearly without without any distortion (whether shrinkage or other dimensional changes) or any notable difference between specimens prepared using Canadian balsam (Fig. 1). Wood micrographs (Fig. 2) from specimens cleared in clove oil and mounted in CNV also did not indicate any difference from those not cleared in clove oil. However, sections prepared in WL varnish were darker (Fig. 4) as they seemed to have retained more safranin stain, but all wood features were clearly seen. It was also observed that the period of setting which could be termed the drying time was far shorter than that of Canadian balsam. The drying time for WL after slide preparation was less than 5 hours as also noted by Kaygin and Akgun, (2008) when used for wood coating. This drying period was as opposed to over 24 to 48 hours for Canadian balsam, while that of CNV was the shortest: it set within one hour. Moreover, specimens could be transferred from alcohol and covered directly with CNV during mounting procedures without clearing a procedure seems not applicable when using Canada balsam as alcohol won't dissolve the Canada balsam.

As seen in Fig. 3, wood features under the light microscope were visibly clear without clearing, an indication that clearing procedure can be by-passed using CNV and that the use of other toxic organic solvents such as xylene can be avoided too. However, the advantage of Canada balsam is the long storage ability of the slides which might not be totally achieved with either CNV and WL. Further investigation should be conducted to ascertain the storage ability of WL. The use of CNV and WL can further eliminate some clear yellow coloration sometimes associated with Canadian balsam after preparation. A coloration which gave an

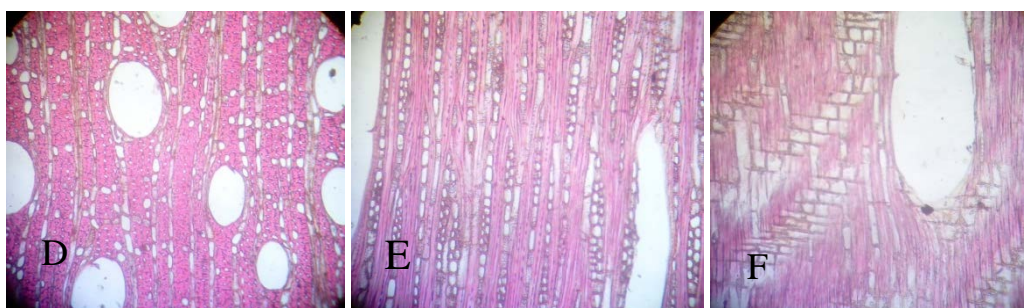
impression of yellowish elements in the sections which could be mistaken for natural deposits in the wood used.

CNV and WL exhibited non-reactivity with the specimens, without darkening or change of refractive index, all of which are qualities of a good mountant. Furthermore, a good mountant should be soluble in the medium in which the specimen was prepared (either aqueous or non-polar, such as xylene or toluene), and not causing the specimen stain to fade or leach. CNV and WL were able to set without cracking or shrinking, and did not shrink back from the edge of cover glass (Lee 1960).



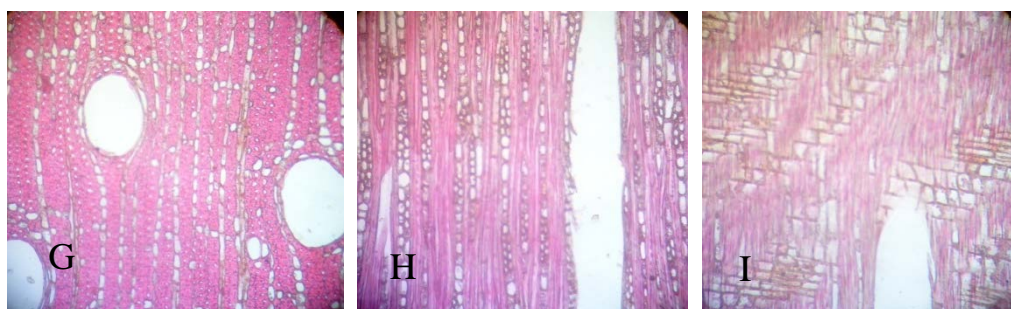
**Fig. 1.**

**Sections mounted in Canadian balsam: A - Transvers section; B - Tangential section; C - Radial section.**



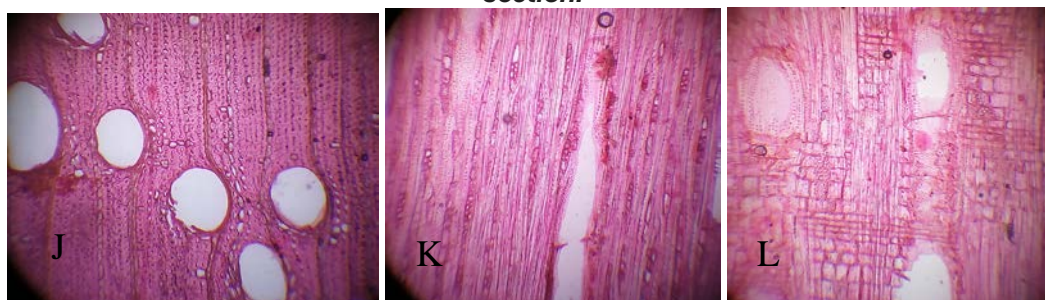
**Fig. 2.**

**Sections mounted in CNV after clearing in clove oil: D - Transvers section; E - Tangential section; F - Radial section.**



**Fig. 3.**

**Sections mounted in CNV without clearing: G - Transvers section; H - Tangential section; I - Radial section.**



**Fig. 4.**

**Sections mounted in WL: J - Transvers section; K - Tangential section; L - Radial section.**

Using WL as a mountant may tend to compete favorably with Canadian balsam even in the area of stability over time because wood varnish can give years of service on outdoor furniture especially when covered with a waterproof material (Williams 1999).

## CONCLUSION

This study has shown that wood varnish and nail varnish can serve as alternative mounting media. The use of clear nail varnish and wood varnish in wood histology will reduce the cost of slide production. Moreover, it is also note-worthy that since clearing can be by-passed through the use of clear nail varnish, plus the fact that drying time for both mountants (nail varnish and wood varnish) is much faster than Canadian balsam will reduce the amount of time required during slide preparation. However, more studies are needed to be carried out in order to ascertain the period of time that specimens can be stored using clear nail varnish and wood varnish.

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