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Replacing DPX Mounting Medium with Natural Oil Alternatives – A Comparative in Vitro Study

Dr.Janhavi Takpire[1]*, Dr.Samruddhi Shinde[1], Dr.Aditi Sangamnere[1], Dr.Shreeja Pillai[2], Dr. Nikita Patel[3], Dr.Nivedita Tayde[4]

[1]Intern, Department of Oral Pathology and Microbiology, SMBT Institute of Dental Sciences and Research, Nandi Hills, Dhamangaon Tal., Igatpuri, Dist, Nashik, Maharashtra 422403, India
[2]Senior Lecturer, Department of Oral Pathology and Microbiology, SMBT Institute of Dental Sciences and Research, Nandi Hills, Dhamangaon Tal., Igatpuri, Dist, Nashik, Maharashtra 422403, India
[3]Senior Reader, Department of Oral Pathology and Microbiology, SMBT Institute of Dental Sciences and Research, Nandi Hills, Dhamangaon Tal., Igatpuri, Dist, Nashik, Maharashtra 422403, India
[4]Senior Lecturer, Department of Oral Medicine and Radiology, SMBT Institute of Dental Sciences and Research, Nandi Hills, Dhamangaon Tal., Igatpuri, Dist, Nashik, Maharashtra 422403, India

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***Corresponding Author**
Dr. Janhavi Takpire

Intern, Department of Oral Pathology and Microbiology, SMBT Institute of Dental Sciences and Research, Nandi Hills, Dhamangaon Tal., Igatpuri, Dist, Nashik, Maharashtra 422403, India

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A B S T R A C T

Introduction*:** A cover slip, also known as cover glass, is a small, thin sheet of glass that is placed over the specimen in a microscope. This is known as mounting. Mounting is the final step in histological preparation of a slide [1]. A tissue specimen must be mounted in order to improve imaging quality during microscopy and to preserve the specimen during storage [2]. The current trend is turning organic, so we tried to discover a natural Dibutyl phthalate xylene (DPX) substitute [3]. DPX is thought to be perfect, yet it has a few disadvantages [4]. The study's objective was to evaluate and compare the effectiveness of DPX as a mounting medium with sandalwood, clove, and cedarwood oils.Aim*:** Compare and evaluate efficacy of natural alternatives with dpx as mounting media.***Materials and Procedure*:** From the archives, four paraffin-embedded blocks with a verified histological diagnosis were removed. From each block, three slices were removed and conventional H&E staining techniques were applied. For every group, three H&E-stained sections and three ground sections were collected. Subsequently, they underwent four distinct mounting media mounts and several parameter analyses [3].***Result*:** The results were tabulated. The Mann-Whitney U test was employed to compare findings. Sandalwood oil demonstrated comparable cellular architecture to D.P.X and other oils, with statistically significant findings for various mounting mediums.***Conclusion:***Because of their distinct properties, sandalwood oil functioned similarly as mounting media to D.P.X. They all have a high refractive index; thus, with few modifications, they can be used a better natural alternative for mounting.

Keywords: DPX, Mounting Medium, Slide, Coverslip, Natural Oils.

INTRODUCTION

Histopathological examination is critical in the diagnosis of many lesions; thus, it must be performed correctly in order to arrive at a final diagnosis. During the course of the treatment plan, the histopathological sections should be evaluated for diagnostic purposes. They must be preserved for a longer amount of time in order to achieve this [5]. Mounting is the final step in histology or pathology laboratories, resulting in a permanent histological preparation on the table following tissue processing and staining [6].

The mounting media is the solution in which the specimen is embedded, typically beneath a cover glass. It can be liquid, gum, or resinous, soluble in water, alcohol, or other solvents, and sealed from the external atmosphere with non-soluble ringing media [7]. A mounting material forms a lasting bond between the slide and coverslip. This protects the cell film against damage, air drying, and stain fading [1]. The ideal mountant should not interfere with the diagnostic

technique and not harm the tissue sections being examined. It should have the same refractive index as the tissue sections and glass slide [8]. Improper immersion media and mounting is a leading source of artifacts being created in the slides prepared.

Mismatching the refractive indices of the mountant and objective immersion medium causes spherical aberration [2]. Refractive index (RI)**,** also called index of refraction, is the measure of the bending of a ray of light when passing from one medium into another and it will help in the proper visualization of structural details of the object [9]. The ideal mounting medium has a refractive index near to 1.5, similar to glass. DPX is the most often used mounting media [10].

A mounting medium should have the following characteristics:

1. A refractive index as close as possible to 1.5.
2. It must be colourless and translucent.
3. The stain should not disperse or fade (the mounting medium used is determined by the staining process).
4. There should be no detrimental effects on cells or tissue components.
5. It should keep the sample safe from physical and chemical activities (such as oxidation).
6. It should set without crystallizing, breaking, or shrinking (to harden mounting media) [10].

Mounting media protects specimens by bonding them to a clear, durable film. The medium plays a crucial role in image production, influencing how the specimen is rendered [8, 11]. The main components of mountants are a base, an antifade reagent, and occasionally a setting plasticizer [12].

There are two types of mounting media: water-based (aqueous, hydrophilic, non-adhesive) and organic solvent-based (resinous, hydrophobic, sticky). Water-based media can be categorized into two types: solidifying media and liquid media. Organic solvents are further categorized into natural and synthetic [6, 12, 13].

DPX is a neutral, colourless medium that preserves the majority of common stains effectively. It is produced by dissolving polystyrene, a common plastic, in an appropriate hydrocarbon solvent (most often xylene) [14].

In addition to its antipyretic, antibacterial, and diuretic features, sandalwood oil plays a significant role in the traditional medical system. Additionally, sandalwood oil has the best fixative properties and possesses a unique stability. Given that sandalwood oil's refractive index ranges from 1.33 to 1.60, which is closer to both glass and DPX refractive indices, it can be considered a natural substitute for DPX [15].

The most popular application for cedarwood oil is in aroma compounds, either as a source of raw materials or as a finished product. It is also utilized in a variety of other goods, including room sprays, disinfectants, soaps, and a few technical treatments. With a refractive index range of 1.48 to 1.51, cedarwood oil is used as a clearing agent for immersion lenses and microscope sections. Its refractive index is comparable to that of DPX and glass, making it a viable substitute for DPX [16].

Clove oil is traditionally used as a flavoring and antimicrobial ingredient in cuisine due to its antibacterial, antifungal, insecticidal, and antioxidant qualities [17-19]. Clove oil has a refractive index between 1.50 and 1.54 [20].

The study's objective was to evaluate and compare the effectiveness of D.P.X. as a mounting medium with sandalwood, clove, and cedarwood oils.

MATERIALS AND METHODS

To evaluate the efficacy of natural alternatives to dpx, four paraffin embedded blocks with verified histopathological diagnosis were sliced into three sections each and three ground sections were taken from department of oral pathology.

Following incubation, the sections were deparaffinized using xylene for 20 minutes, and then the standard procedure for Hematoxylin and eosin staining was completed. After staining, the slides were cleaned and prepared for mounting.

For every group, three H&E-stained sections and three ground sections were collected. Subsequently, they underwent four distinct mounting media mounts and several parameter analyses [3].

They were categorised as:

GROUP A	DPX
GROUP B	SANDALWOOD OIL
GROUP C	CEDARWOOD OIL
GROUP D	CLOVE OIL

The scores were based on the clarity of cellular details, the presence of air bubbles, the adhesive of the coverslip and glass slide, and transparency.

Score 0,1, 2, 3 for no, mild, good, and very good clarity of cellular details, respectively.
Score 0, 1,2,3 for absence and presence of air bubbles.
Score 0, 1, 2, 3, for no, mild, good, and very good adhesion.
Score 0, 1, 2, 3, for no, mild, good, and very good transparency.

Statistical Test: The Mann-Whitney U test was employed to compare findings.

RESULTS

Based on the median values provided by the observer, comparable cellular architecture was shown by sandalwood oil compared to D.P.X and other oils, with findings that were statistically significant for different mounting media.

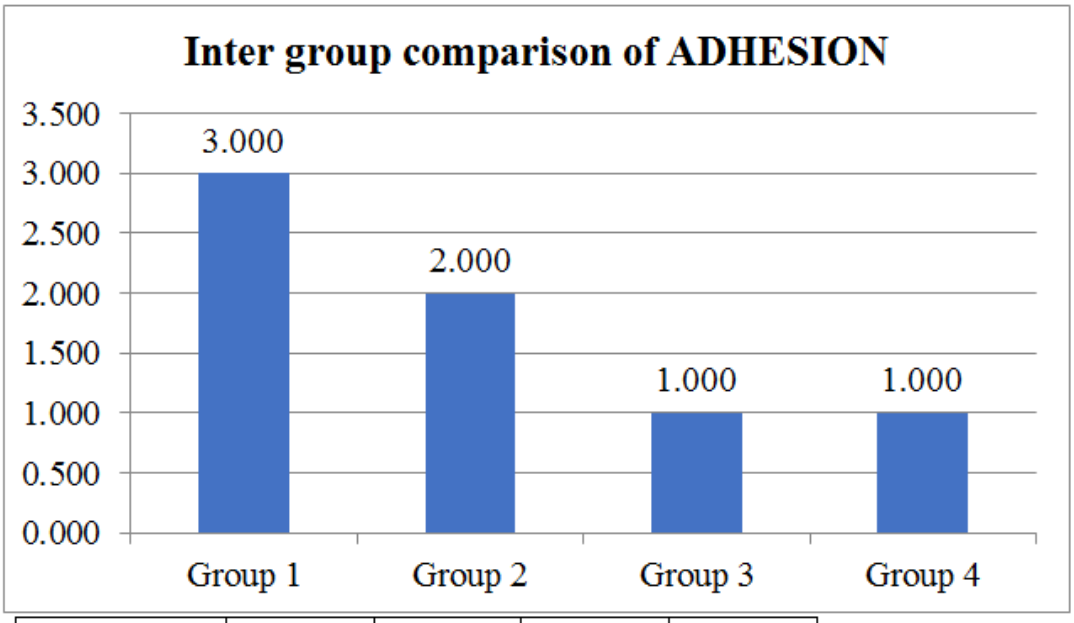
A statistically significant difference ($p<0.01$) was seen in the values of ADHESION across the groups, with greater values in Group 1.

CLARITY OF CELLULAR DETAILS in Group 1 with higher scores.
AIR BUBBLE PRESENCE, with greater values in groups 3 and 4.
TRANSPARENCY in group 1 with greater values.

Groups are ranked as:

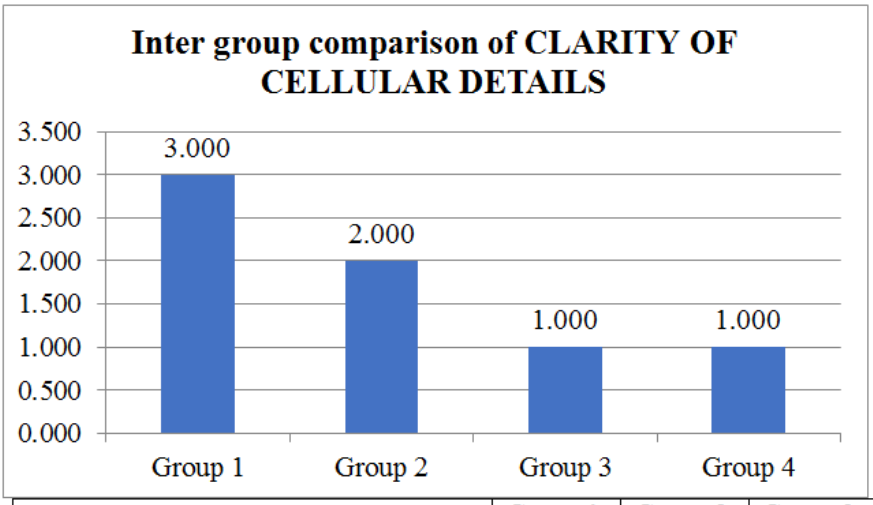
1.	DPX
2.	SANDALWOOD OIL
3.	CEDARWOOD OIL
4.	CLOVE OIL

ADHESION



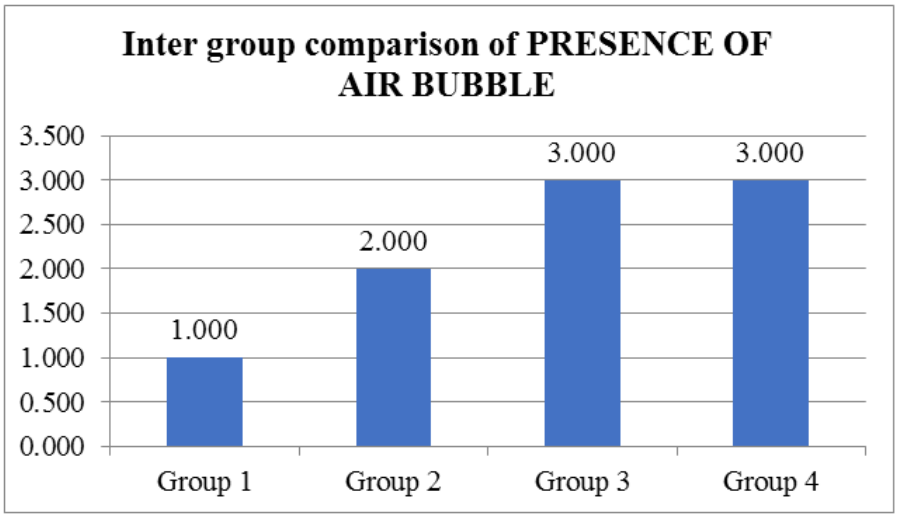
	Group 1	Group 2	Group 3	Group 4
ADHESION	3.000	2.000	1.000	1.000

CLARITY OF CELLULAR DETAILS



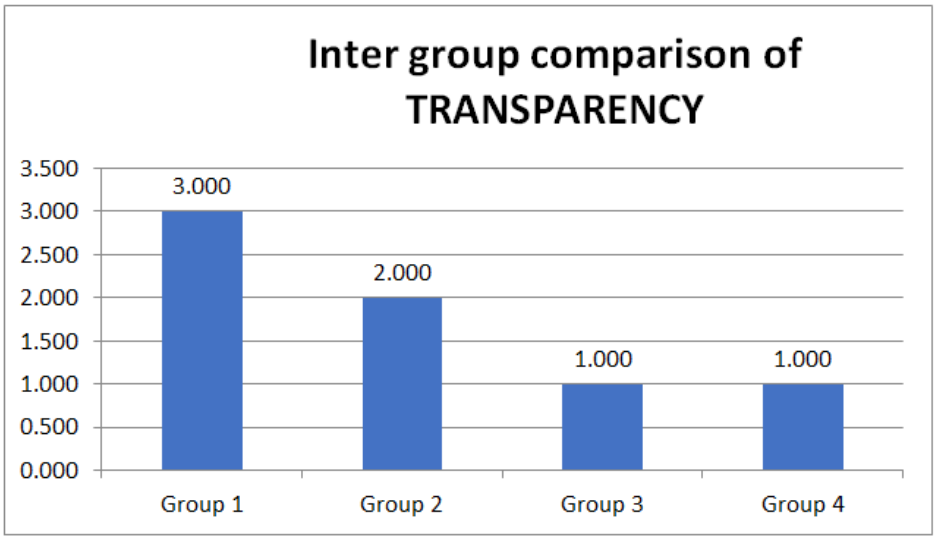
	Group 1	Group 2	Group 3	Group 4
CLARITY OF CELLULAR DETAILS	3.000	2.000	1.000	1.000

PRESENCE OF AIR BUBBLE

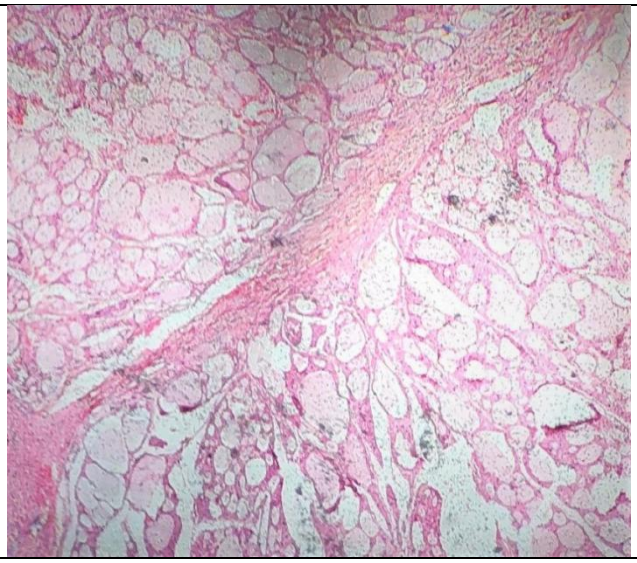


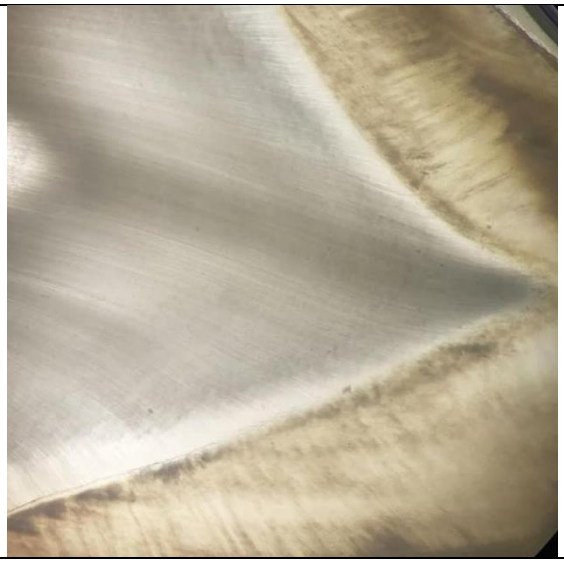
	Group 1	Group 2	Group 3	Group 4
PRESENCE OF AIR BUBBLE	1.000	2.000	3.000	3.000

TRANSPARENCY

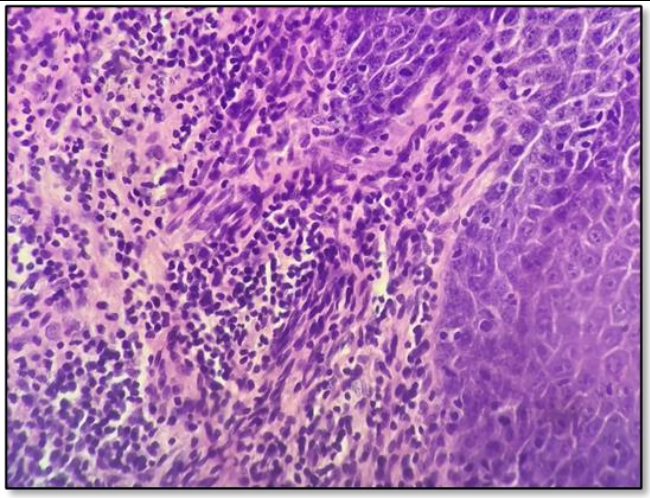


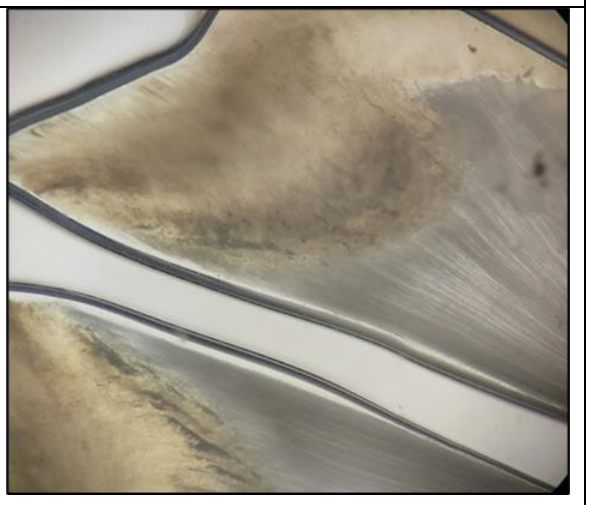
	Group 1	Group 2	Group 3	Group 4
TRANSPARENCY	3.000	2.000	1.000	1.000



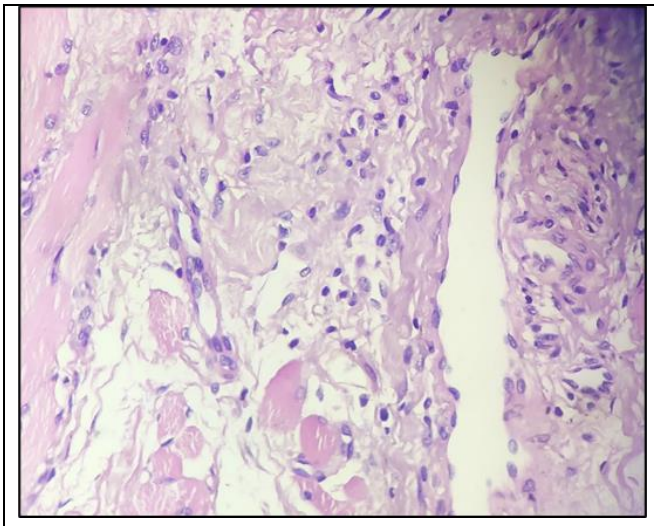


DPX

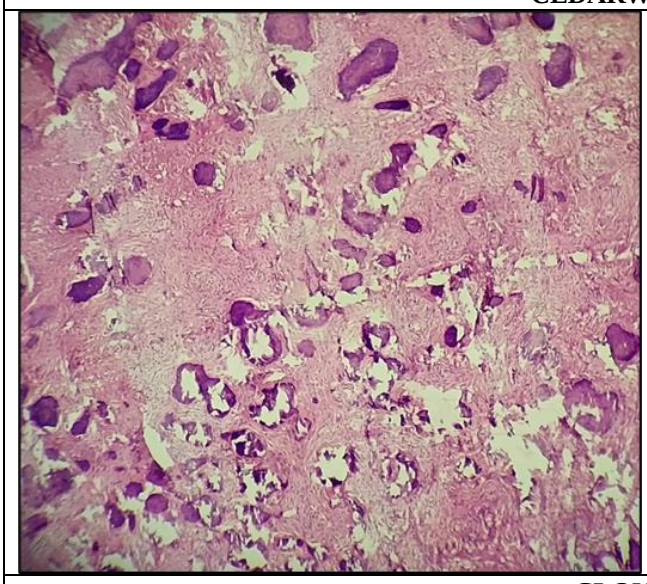
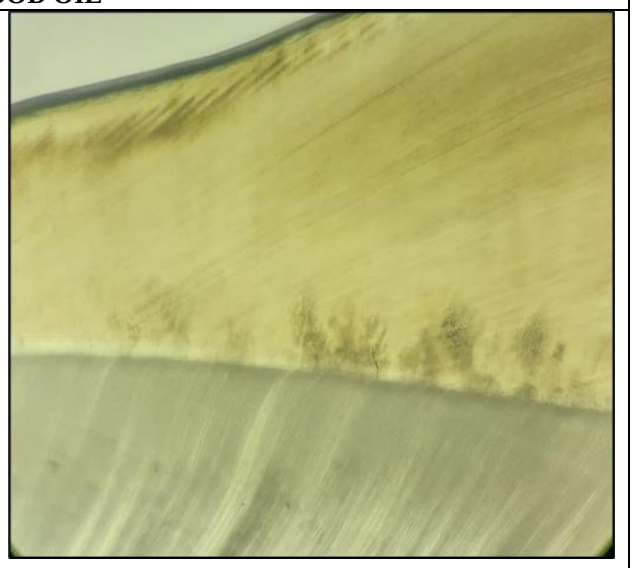




SANDALWOOD OIL




CEDARWOOD OIL

CLOVE OIL

DISCUSSION

Mounting, the final stage, is crucial to preserving the sections for a long period and producing an accurate image under a microscope [5, 8, 21]. When selecting the appropriate mounting media, a number of aspects need to be taken into account. These include things like toxicity, refractive index, compatibility with the specimen, pigment stability, shrinkage, durability, affordability, and convenience of usage [24].

To provide maximum transparency, mounting material with a refractive index similar to glass slides (1.51-1.52) is usually used [22].Mounting media should not shrink or cause the stain to diffuse or fade. It should be colourless, transparent, dry, and harden rapidly [6].

The histopathological section should be sealed off from the outside environment to prevent damage to the specimen physically. Without interfering with the process, mounting media adhere the specimen to a cover slip on a glass slide displaying distinct cellular features [8].

The following reagents are needed to prepare the DPX mountant:

1. Polystyrene (Distrene 80) 18g
2. Dibutyl Phthalate .....7.5 ml
3. Xylene .....52.5 ml

The refractive Index of the solution is 1.523 [23]

The main disadvantage of polystyrene medium is its quick setting and tendency to withdraw from the cover slip edge. A plasticizer can inhibit mesh formation with polyunsaturated plastics [14].

DPX is a universal mountant that meets most of the requirements for an ideal mountant. However, despite its many benefits, it has some drawbacks, such as a longer setting time (it takes longer to set) and potentially dangerous effects due to the presence of xylene and dibutyl phthalate in its main constituent [3]. D.P.X.'s adverse consequences include teratogenicity, irritation of the eyes, skin, and respiratory tract, aspiration hazard if eaten, potential for lung injury if absorbed via the skin, and potential for central nervous system depression [25].

Even tissue dehydration is necessary for DPX. As is well known, anyone who will be handling this, such as laboratory workers, pathologists, and scientists working in the laboratory, may be exposed to chemicals used in the mounting procedure. Therefore, it's essential to develop biodegradable and healthful options in the industry [3].

Oil has shown to be a higher-quality mounting material than DPX [1].

As an alternative to commonly used mountants, organic compounds including sandalwood oil, cedarwood oil, and clove oil were also used in this study. While sandalwood oil and D.P.X produced comparable levels of clarity and transparency for cellular details, we found that clove oil and cedarwood oil produced lower levels of cellular details and transparency, though they have refractive index similar to glass slide and tissue section.

Sandalwood oil has a pleasant smell and good handling properties. Clove oil and cedarwood oil have poor handling properties as well as smell. In addition, naturally occurring materials like sandalwood, cedarwood, and clove oils were used in this study as substitutes for widely used mountants. As we can see from the pictures, sandalwood oil created good cellular detail clarity and transparency comparable to D.P.X. While clove oil discoloured the slide yellow and had poor adhesion, cedarwood oil had poor cellular clarity that made it difficult to appreciate the cells.

Based on earlier research on castor oil and honey, castor oil was found to have a few superior characteristics than DPX; yet, in this particular examination, DPX outperformed the other oils tested [3]. DPX has superior qualities in comparison to oils.

CONCLUSION

Sandalwood oil showed comparable clarity and transparency to D.P.X as a mounting medium. However, cedarwood and clove oil did not adhere to the slide cover slip but can be used as an alternative with modifications. Clove oil and cedarwood oil had weak cellular features but a high refractive index. Sandalwood oil has an appealing aroma when compared to the other oils. Cedarwood oil has an awful odour, which makes handling difficult, and adhesion is likewise poor. Clove oil has a strong odour, showed yellowish staining on the slides, so cellular clarity is not appreciated adequately.

According to the results, they are ranked as follows: DPX, sandalwood oil, cedarwood oil, and clove oil. In the future, with certain improvements, they can be considered as an alternative to DPX.

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