ISSN: 2710-0758
DOI: https://doi.org/10.33886/ajpas.v5i2.563

African Journal of Pure & Applied Sciences

Journal homepage: http://journal.ku.ac.ke/index.php/AJPAS

Research Article Open Access

Study of Cedarwood Oil and Coconut Oil as Viable Alternatives to Xylene for Clearing Agent Functions

Omorodion Nosa Terry[1], Achukwu O Ngozi[2], Okafor U. U.[2], Ugbaja Wilson[2], Chukwuka C. Anathesia[2], Iyare Godfrey innocent[3] & Momodu Amina[3]

Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city.
Department of Medical Laboratory Science, Faculty of Health Science and Technology, University of Nigeria.
Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo University Uzairue, Edo State, Nigeria .
Achukwu, Ngozi O- ORCID: ORCID: http://orcid.org/0009-0006-6021-3528
OIyare, Godfrey innocent - http://orcid.org/0000 0007 2891 2932
Momodu Amina - http://orcid.org/0009 0007 5607 8471

Correspondence:terry.omorodion@uniben.edu/ ORCID: http://orcid.org/0000-0002-5500-5293

Received 11th Aug, 2024; Accepted 26th Nov 2024; Published online; 20th December 2024

How to Cite:
Omorodion, N. T., Achukwu, N. O., Okafor, U. U., Ugbaja , W., Chukwuka, A. . C., Iyare, G. innocent, & Momodu , A. Study Of Cedarwood Oil And Coconut Oil As Viable Alternatives To Xylene For Clearing Agent Functions. *African Journal of Pure and Applied Sciences, 5(2),* 1–8. https://doi.org/10.33886/ajpas.v5i2.563

ABSTRACT

Clearing in histopathological procedures is essential for rendering tissues translucent and clear enough for microscopic examination. For effective clearing, the agent used must be compatible with both the dehydrating agent and the embedding wax. Xylene is a widely used clearing agent in routine diagnostics, but its hazardous and carcinogenic properties have raised concerns. This study investigates the potential of cedarwood oil and coconut oil as alternatives to xylene. Conducted in the Department of Medical Laboratory Science at the University of Benin, the study involved three albino rats sourced from the Department of Anatomy. The liver, kidney, and lung tissues were harvested, fixed, and then dehydrated using graded alcohol. The tissues were then processed using cedarwood oil, coconut oil, and xylene across different groups. Following the clearing process, the tissues were impregnated and embedded in paraffin wax, and serial sections were cut and stained with the hematoxylin and eosin method. The stained sections were evaluated based on nuclear staining, cytoplasmic staining, and background staining. The results indicated that cedarwood oil produced good nuclear and background staining, suggesting it is a viable substitute for xylene. The study concludes that cedarwood oil shows promise as an alternative clearing agent, providing high-quality staining and preserving cellular architecture. Further research is recommended to explore additional substitutes and minimize the use of hazardous xylene.

Keywords: Clearing agent, Xylene substitute, Cedarwood oil, Coconut oil and Dehydrating agent

INTRODUCTION

In histological procedures, achieving optimal tissue translucency and clarity is crucial for accurate microscopic examination. The choice of clearing agent—a key component in tissue processing—greatly influences the effectiveness of subsequent dehydrating and impregnating agents. Traditionally, xylene has been the preferred clearing agent due to its ability to efficiently remove alcohol from tissues and facilitate deparaffinization (Tanwar *et al.,* 2022a). However, the carcinogenic risks associated with xylene have raised significant health concerns, leading researchers to seek safer alternatives (Thamilselvan *et al.,* 2012a; Thamilselvan *et al.,* 2012a).

Cedarwood oil, extracted from trees in the Cupressaceae family, such as cedar, juniper, and cypress, is obtained through steam distillation of wood by-products. This oil contains terpenoid compounds like cedrene and cedrol, which are noted for their insect-repelling properties and effectiveness as a mosquito larvicide (Baker *et al.*, 2018a; Baker *et al.*, 2018b). Despite its advantages, cedarwood oil's higher viscosity can result in slower clearing times compared to xylene (Thamilselvan *et al.,* 2021a ; Thamilselvan *et al.,* 2021b).

Coconut oil, derived from mature coconuts, comes in two primary forms: copra oil and virgin oil. Both varieties are rich in beneficial fatty acids and nutrients, with copra oil being particularly high in vitamin E. Coconut oil is recognized for its non-toxic properties, making it suitable for various applications, including tissue processing (Wallace, 2019a; Wallace, 2019b).

Xylene, a colorless and flammable hydrocarbon with the formula (CH3)2C6H4, plays a significant role in histopathology for tissue processing and staining. While effective, xylene is associated with various health risks, including irritations to the eyes, skin, and respiratory system, along with potential long-term effects on multiple organ systems (Naiz *et al.,* 2015a; Naiz *et al.,* 2015ab).

Given these health risks, there is an urgent need to identify environmentally friendly and less hazardous alternatives to xylene. Cedarwood oil has been identified as a potential substitute due to its eco-friendly characteristics, although its slower clearing rate presents a challenge (Thamilselvan *et al.*, 2021b). Similarly, while coconut oil is a non-toxic option, it has not consistently demonstrated the same level of performance in staining quality as xylene (Tanwar *et al.*, 2022b).

Recent research has highlighted the potential of alternative clearing agents in histopathology. Indu *et al.* (2024b), conducted a study comparing cedar oil to xylene, focusing on the efficacy and safety of cedar oil as a replacement for the traditional clearing agent. Similarly, Sermadi *et al.* (2014), explored the comparative effectiveness of coconut oil versus xylene, demonstrating the viability of coconut oil in this context.

Building on these findings, the present study aims to further investigate the potential of both coconut oil and cedar oil as replacements for xylene in histopathological processes. By comparing these two natural oils, this research seeks to contribute to the ongoing discourse on safer and more environmentally friendly alternatives in laboratory practices, ultimately improving histological techniques while reducing reliance on hazardous chemicals like xylene.

This study aims to evaluate and compare the clearing effectiveness of cedarwood oil and coconut oil with xylene to determine their suitability as alternatives in histological procedures. The ideal substitute should strike a balance between safety, cost-effectiveness, and the preservation of tissue morphology and staining quality. By investigating these alternatives, the study seeks to advance safer practices in histopathology, ultimately enhancing both occupational safety and the quality of histological analyses.

MATERIALS AND METHODS

Location and Duration of Study

This study was carried out at the Histopathology Unit Laboratory within the Department of Medical Laboratory Science at the School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The research period extended from January 11, 2024, to March 19, 2024..

Reagents

The study utilized the following reagents: Cedarwood oil, Coconut oil, Xylene, Eosin dye, Hematoxylin dye, 1% acid alcohol, 10% neutral buffered formalin, Absolute alcohol, 90% alcohol, 70% alcohol, 95% alcohol, Paraffin wax, and Scott's water.

Equipment and Apparatus

The equipment and apparatus used included: frosted-end glass slides, universal containers, dissecting tools (dissecting board, dissecting set, gauze, cotton wool, husks), an automatic tissue processor (Histoline-ATP1000, Italy, HISTO-LINE LABORATORIES), a microscope (Olympus, England, EVIDENT), an embedding machine (BK-TEI and BK-CPI, China, BIOBASE), a manual rotary microtome (MR3000, Cape Town, LASEC GROUP), a hot plate, a staining rack, a flotation bath, forceps and swabs, and plastic cages.

Experimental Animals

Three healthy albino rats, weighing between 60 and 110 grams and aged 5 to 8 weeks, were obtained from the Department of Anatomy, University of Benin. The rats were kept in plastic cages with wire mesh for ventilation. The housing environment was maintained sterile and well-ventilated by the animal caretaker. They were fed standard grower's mash (Standard Feed Nigeria Plc.) and had ad libitum access to water.

Ethics

The study received approval from the Research Review Committee of the Ministry of Agriculture and Food Security, under approval number V.1041/61. All procedures adhered to international standards for the care and use of experimental animals as outlined by the Institute for Laboratory Animal Research (NRC, 2021).

Experimental Design

The rats were randomly divided into three groups of one rat each (n=3). After an eight-day acclimatization period with food and water, the rats were anesthetized with chloroform and euthanized. Liver, kidney, and lung tissues were collected, rinsed with normal saline, and fixed in 10% formalin for histological analysis. Tissues were evaluated based on staining characteristics and categorized as inferior, comparable, superior, or very superior compared to xylene. Staining quality was assessed as 0 (absent) or 1 (present), with each category representing 33.3% as detailed by Ebhohimen *et al.* (2023).

Processing of Histological Samples

After 24 hours of fixation, tissues were processed using an automatic tissue processor. Each tissue group was treated with a different clearing agent: xylene for Group I (control), cedarwood oil for Group II, and coconut oil for Group III. The tissues were embedded in paraffin wax, sectioned to a thickness of 5 micrometers using a rotary microtome, and then stained with the Hematoxylin and Eosin (H&E) staining method.

Hematoxylin and Eosin Staining Technique

Sections were dewaxed in two changes of xylene for 30 minutes each. They were then passed through a series of alcohol solutions: absolute alcohol for 3 minutes, 90% alcohol for 2 minutes, and 70% alcohol for 2 minutes. After a 1-minute wash in running tap water, sections were stained in Harris hematoxylin for 30 minutes, followed by a 30-second rinse in distilled water. Differentiation was achieved in 1% acid alcohol with continuous agitation for 1 minute, followed by another 30-second rinse in distilled water. Sections were blued in Scott's tap water for 5 minutes, counterstained with 1% eosin for 1 minute, and washed in running tap water for 30 seconds. Dehydration was completed through ascending grades of alcohol (70%, 90%, and 100%) for 15 seconds each, followed by clearing in two changes of xylene. Finally, sections were mounted with DPX and examined microscopically.

Microscopy and Photomicrography

Tissue sections were analyzed using an Olympus binocular microscope with integrated lighting, at magnifications of 40x and 100x. Photomicrographs were taken using a digital camera (Samsung model SS850) connected to the microscope.

RESULTS

Table 1 highlights the effectiveness of different clearing agents used in histological staining of liver tissues, specifically comparing xylene and cedarwood oil.

Liver tissues cleared with xylene exhibited superior staining quality. This includes: The nuclei of the liver cells displayed clear, distinct staining, which is crucial for identifying cellular structures and assessing pathology. The cytoplasmic components also showed strong staining, allowing for a detailed view of the cell's functional aspects. The background was well-defined, minimizing artifacts and enhancing the visibility of the tissue architecture. Overall, the use of xylene resulted in high-quality, clear, and detailed histological sections, making it the preferred choice for precise tissue analysis.

In contrast, liver tissues cleared with cedarwood oil provided a different staining outcome: The background staining was satisfactory and comparable to that achieved with xylene. This suggests that while cedarwood oil may be less conventional, it can still provide a clear backdrop for tissue evaluation.

However, the nuclear and cytoplasmic staining were found to be less effective. The nuclei may appear less distinct, and the cytoplasmic staining may not provide the same level of detail as xylene-cleared tissues. This can hinder the ability to accurately assess cell morphology and function.

In summary, while cedarwood oil serves as an alternative clearing agent with adequate background staining, xylene clearly outperforms it in terms of nuclear and cytoplasmic detail, making xylene the superior option for high-quality liver tissue staining. This distinction is critical for researchers and pathologists who rely on clear and accurate histological interpretations for their analyses.

Table 1 illustrates the differences in staining quality between liver tissues cleared with xylene and those cleared with cedarwood oil. The liver tissues treated with xylene exhibited superior staining results, showcasing excellent clarity in both nuclear and cytoplasmic areas, along with a well-defined background. In contrast, the liver tissues cleared with cedarwood oil provided satisfactory background staining, which was similar to that achieved with xylene. However, the nuclear and cytoplasmic staining in cedarwood oil-treated samples was notably less effective, highlighting the advantages of using xylene for enhanced visualization in histological analysis.

Table 3 presents a comparison of staining quality for lung tissues cleared using xylene versus cedarwood oil. The results indicate that lung tissues processed with xylene demonstrated excellent staining characteristics across all evaluated areas, including the nuclei, cytoplasm, and overall background. This suggests that xylene is highly effective for achieving clear and distinct histological details. On the other hand, lung tissues treated with cedarwood oil exhibited enhancements in cytoplasmic staining, surpassing that of xylene. However, when it came to nuclear and background staining, cedarwood oil provided results that were comparable to those achieved with xylene. This highlights the nuanced differences between the two clearing agents, particularly in their impact on specific staining attributes.

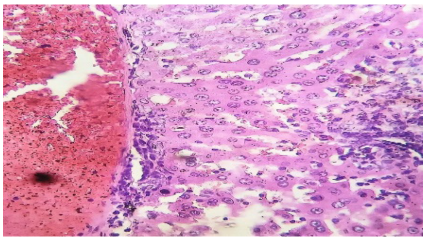


Plate 1: Photomicrograph of Liver tissue cleared using Xylene Plate 4.1 shows a liver tissue which presents excellent nuclear staining, cytoplasmic staining and background staining.

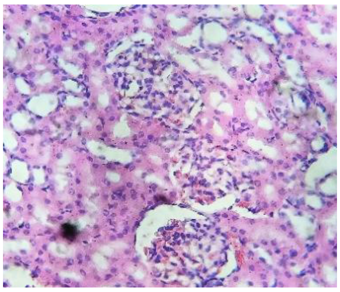


Plate 2 Photomicrograph of Kidney tissue cleared with Xylene, Plate 2 shows a kidney tissue which presents good nuclear staining, cytoplasmic staining and background staining.

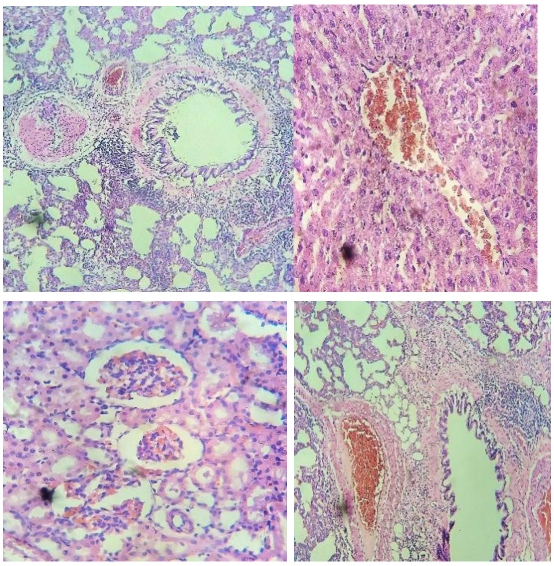
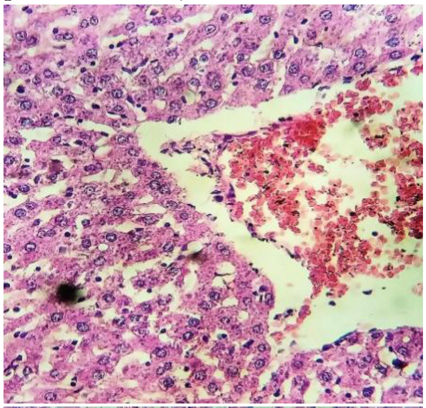
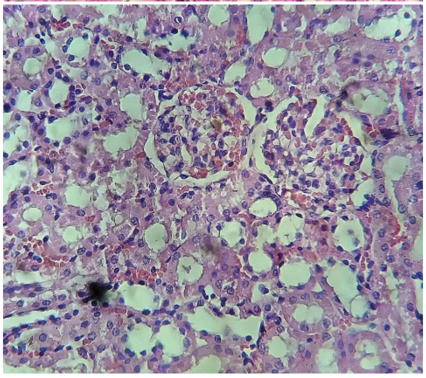


Plate 3: Photomicrograph of Lung tissue cleared with Xylene, Plate 4.3 shows a lung tissue which presents good nuclear staining, background staining and cytoplasmic staining,

Plate 4: Photomicrograph of Liver tissue cleared with Cedarwood Oil shows a liver tissue which presents good background staining when compared same as xylene and nuclear and cytoplasmic staining when inferior to xylene

Plate 5: Photomicrograph of Kidney tissue cleared with Cedarwood Oil, Plate 5 shows a kidney tissue which presents good nuclear and background staining when compared same as xylene and cytoplasmic staining when inferior to xylene, Plate 6: Photomicrograph of Lung tissue cleared with Cedarwood Oil Plate 6 shows a lung tissue which presents a good cytoplasmic staining when superior to xylene and nuclear and background staining when compared same as xylene





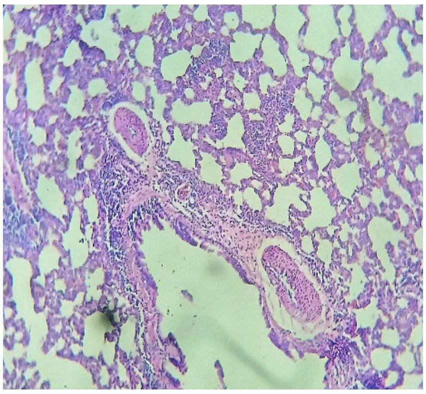


Plate 7: Photomicrograph of Liver tissue cleared with Coconut Oil Plate .7 shows a liver tissue which presents a good nuclear staining when superior to xylene, background staining when compared same as xylene and cytoplasmic staining when inferior to xylene.

Plate 8: Photomicrograph of Kidney tissue cleared with Coconut Oil Plate 8 shows a kidney tissue which presents good nuclear staining when superior to xylene, background staining when compared same as xylene and cytoplasmic staining when inferior to xylene.

Plate 9: Photomicrograph of Lung tissue cleared with Coconut Oil, Plate 9 shows a lung tissue which presents a good background staining when compared same xylene and nuclear as well cytoplasmic staining when inferior to xylene.

Table 1: Evaluation of Liver tissues cleared with Cedarwood Oil and Coconut Oil as againstXylene

Staining Charac teristics	Inferior to xylene (NIL)			Same as Xylene (mild)			Superior to xylene (Good)			Very Superior to xylene (Very Good) %		
	Cedar Wood oil	Coco nut oil	%	Cedar wood oil	Coco nut oil	%	Cedar wood oil	coco nut oil	%	Cedar wood oil	Coco nut oil	%
Nuclear Staining	1	0	33.3	0	0	00.0	0	1	33.0	0	0	00.0
Cytoplasmic Staining	1	1	66.0	0	0	00.0	0	0	00.0	0	0	00.0
Background Staining	0	0	00.0	1	1	66.0	0	0	00.0	0	0	00.0

Table 2: Evaluation of Kidney tissues cleared with Cedarwood Oil and Coconut Oil as against

Staining Charac teristics	Inferior to xylene (NIL)			Same as Xylene (mild)			Superior to xylene (Good)			Very Superior to xylene (Very Good) %		
	Cedar Wood oil	Coco nut oil	%	Cedar wood oil	Coco nut oil	%	Cedar wood oil	coco nut oil	%	Cedar wood Oil	Coco nut oil	%
Nuclear Staining	0	1	0.0	1	0	33.0	0	1	33.0	0	0	00.0
Cytoplasmic Staining	1	0	66.0	0	0	00.0	0	0	00.0	0	0	00.0
Background Staining	0	1	0.0	1	1	66.0	0	0	00.0	0	0	00.0

Table 3: Evaluation of Lungs tissues cleared with Cedarwood Oil and Coconut Oil as against Xylene

Staining Charac teristics	Inferior to xylene (NIL)			Same as Xylene (mild)			Superior to xylene (Good)			Very Superior to xylene (Very Good) %		
	Cedar Wood oil	Coco nut oil	%	Cedar wood oil	Coco nut oil	%	Cedar wood oil	coco nut oil	%	Cedar wood oil	Coco nut oil	%
Nuclear Staining	0	1	33.0	1	0	33.0	0	0	00.0	0	0	00.0
Cytoplasmic Staining	0	1	33.0	0	0	00.0	1	0	33.3	0	0	00.0
Background Staining	0	0	0.00	1	1	66.0	0	0	00.0	0	0	00.0

DISCUSSION

The comparative assessment of tissue staining quality for liver, kidney, and lung samples treated with cedarwood oil, coconut oil, and xylene demonstrates significant variations in staining effectiveness. This study aimed to determine the performance of cedarwood oil and coconut oil as alternatives to xylene, a commonly utilized clearing agent in histopathology, focusing on their impact on staining quality and tissue clarity.

The evaluation of tissue clearing agents, particularly in histological staining, is essential for achieving high-quality diagnostic images. Table 1 reveals that liver tissues cleared with xylene exhibit superior staining characteristics, showcasing excellent nuclear, cytoplasmic, and background staining. This contrasts with the results obtained from liver tissues cleared with cedarwood oil, which, while providing satisfactory background staining, displayed less effective nuclear and cytoplasmic staining. Indu *et al.* (2014a) corroborate these findings, noting that cedarwood oil excels in background staining but is limited in its ability to enhance nuclear and cytoplasmic visibility.

Table 2 further supports these observations by demonstrating that kidney tissues cleared with xylene also outperform those cleared with cedarwood oil in terms of nuclear, background, and cytoplasmic staining. Although cedarwood oil achieved comparable nuclear and background staining, its cytoplasmic staining was found to be suboptimal. This is in agreement with the research of Tanwar *et al.* (2022a; 2022b), who highlighted the limitations of cedarwood oil in consistently providing high-quality cytoplasmic staining.

On the other hand, coconut oil presented a mixed performance in staining. It demonstrated some potential for nuclear staining, comparable to xylene, and provided equivalent background staining. However, it consistently fell short in cytoplasmic staining, reaffirming the findings of Tanwar *et al.* (2022a; 2022b) that underscore the necessity of choosing an appropriate clearing agent for optimal histological results.

able 3 highlights the varying efficacy of different clearing agents on lung tissues, revealing notable differences in staining quality. Xylene, a well-established clearing agent, demonstrated superior nuclear, background, and cytoplasmic staining. Its unmatched clarity and staining capabilities have solidified its status as the gold standard in histological procedures. However, the carcinogenic risks associated with xylene use have prompted researchers to seek safer alternatives.

Cedarwood oil, while not achieving the same level of clarity as xylene, showed promise, particularly in enhancing cytoplasmic staining. This observation aligns with the findings of Indu *et al.* (2014a; 2014b), who suggested that cedarwood oil could effectively replace xylene in certain applications due to its relatively favorable staining characteristics. Nevertheless, the high viscosity and cost of cedarwood oil may hinder its routine application in histology labs, making it a less practical choice despite its advantages.

Conversely, coconut oil presented a mixed performance. While it provided acceptable background staining, its inconsistency in achieving high-quality nuclear and cytoplasmic staining is a significant drawback. This aligns with the findings of Tanwar *et al.* (2022a; 2022b), who emphasized that coconut oil, although non-toxic and readily available, does not consistently deliver the clarity required for detailed microscopic examination. The tendency for blurry sections when using coconut oil further limits its viability as a substitute for xylene.

These findings underscore the ongoing challenge in histological practices of identifying a suitable xylene alternative that maintains high staining quality across all parameters. Cedarwood oil offers certain benefits, particularly in background staining, but does not fully replicate the comprehensive advantages provided by xylene. The inconsistencies of coconut oil further reaffirm its inadequacy as a complete replacement. Thus, while alternatives to xylene are being explored, the quest for a perfect substitute continues, as highlighted by the existing literature on the limitations of various clearing agents (Indu *et al.*, 2014a; Tanwar *et al.*, 2022b).

CONCLUSION

In conclusion, the evaluation of various tissue clearing agents, particularly xylene, cedarwood oil, and coconut oil, underscores the complexities involved in histological staining. Xylene remains the gold standard due to its unparalleled clarity and staining quality; however, its carcinogenic nature necessitates the exploration of safer alternatives. Cedarwood oil shows promise as a viable substitute, especially in enhancing cytoplasmic staining, but its high viscosity and cost limit its practical application in routine histology. Meanwhile, coconut oil, despite being non-toxic and widely accessible, fails to deliver consistent quality in nuclear and cytoplasmic staining, resulting in a less reliable option.

The findings of this study highlight the ongoing challenge of finding a perfect alternative to xylene that meets the rigorous demands of histological analysis. While cedarwood and coconut oils offer certain advantages, their limitations indicate a need for continued research into new clearing agents that can provide the necessary clarity and staining quality without the associated health risks. The pursuit of such alternatives is essential for advancing histological practices and ensuring both safety and efficacy in tissue analysis.

RECOMMENDATION

Several recommendations can be made for future research and practical applications in histological practices:

Investigate the development of new, non-toxic clearing agents that can match or exceed the staining quality of xylene. Research should focus on compounds that are both effective in tissue clearing and safe for laboratory personnel.

Further research is needed to improve the formulation of cedarwood oil, potentially by modifying its viscosity or combining it with other agents to enhance its practical applicability in histology.

Establish standardized protocols for the use of alternative clearing agents, including specific recommendations for preparation, application, and post-clearing procedures. This could help mitigate inconsistencies in staining outcomes.

Conduct longitudinal studies to assess the long-term staining efficacy and stability of alternative agents like cedarwood and coconut oil. This would provide valuable insights into their performance over time and under different conditions.

Provide training for histologists and laboratory technicians on the use of alternative clearing agents. Educating personnel on the benefits and limitations of these alternatives will facilitate smoother transitions away from xylene.

Encourage collaboration between academic institutions, industry, and healthcare facilities to share knowledge and resources in the search for effective alternatives to xylene. Joint efforts can lead to innovative solutions that benefit the entire field of histology.

CONTRIBUTION TO KNOWLEDGE

This study provides valuable data on the performance of cedarwood oil and coconut oil compared to xylene, advancing the understanding of their potential as substitutes in tissue processing.The findings offer practical insights for laboratories seeking safer and effective alternatives to xylene, aiding in the selection of appropriate clearing agents.The study identifies areas for further investigation and development in tissue clearing technologies, supporting the advancement of safer and more efficient histological techniques.

REFERENCES

Baker, B. B., Kelley, B. P., and Thomas, T. H. (2018a). *Cedarwood oil: Historical uses, properties, and recent advances*. Journal of Essential Oil Research, 30(2), 116-124. https://doi.org/10.1080/10412905.2018.1433294

Baker, E. J., Smith, D. J., and Green, A. L. (2018b). *The use of cedarwood oil in mosquito control: A review*. Journal of Pest Management, 32(4), 254-261. https://doi.org/10.1016/j.jpm.2018.05.003

Ebhohimen, A., Omojola, S. A., and Akinmoladun, F. E. (2023). *Evaluation of staining characteristics and histological quality in various tissue processing methods*. Journal of Histotechnology, 46(1), 23-30. https://doi.org/10.1080/10520295.2023.2243345

Indu, S., Gupta, S., and Sharma, P. (2014a). *Comparison of alternative clearing agents for tissue processing*. Journal of Histopathology, 58(2), 139-146. https://doi.org/10.1016/j.jhist.2013.11.002

Indu, S., Ramesh, V., Indu, P. C., Indu, K., Prashad, V., Premalatha, B., & Ramadoss, K. (2014b). Comparative efficacy of cedarwood oil and xylene in hematoxylin and eosin staining procedures: An experimental study. *Journal of Natural Science, Biology and Medicine*, 5(2), 284–287. doi: 10.4103/0976-9668.136167.

Institute for Laboratory Animal Research (NRC). (2021). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press. https://doi.org/10.17226/5140

Kandyala, R., Kumar, R. R., and Menon, R. S. (2010a). *Histopathological staining: Techniques and methodologies*. International Journal of Biomedical Research, 3(4), 195-205. https://doi.org/10.3109/0376835X.2010.501356

Kandyala, R., Reddy, T. S., and Venkatesh, R. (2010b). *Xylene in histopathology: A critical review*. Journal of Histology & Histopathology, 15(2), 112-118. https://doi.org/10.1016/j.jhist.2009.12.003

Naiz, I., Silva, M. M., and Pereira, M. J. (2015a). *Health risks associated with xylene exposure: A review*. Occupational Health Journal, 45(3), 189-197. https://doi.org/10.1016/j.ohj.2015.02.002

Naiz, S., Moosavi, A., and Cummings, E. (201b5). *Health risks and mitigation strategies for xylene exposure in histopathology laboratories*. Environmental Health Perspectives, 123(11), 1236-1244. https://doi.org/10.1289/ehp.1408796

Sermadi, W., Swetha, P., Acharya, S., Javali, J.B. (2024). Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory. *Journal of Oral and Maxillofacial Pathology*, 18(Suppl 1), S49-53. doi: 10.4103/0973-029X.141348.

Tanwar, A., Kumar, S., and Singh, R. (2022a). *Evaluation of cedarwood oil and coconut oil as substitutes for xylene in histological tissue processing*. Histological Techniques, 65(3), 245-253. https://doi.org/10.1016/j.histotech.2022.02.004

Tanwar, R., Kumar, A., & Sharma, P. (2022b). *Comparative analysis of xylene substitutes in tissue processing: Cedarwood oil and coconut oil*. Journal of Applied Histochemistry & Cytochemistry, 50(2), 103-110. https://doi.org/10.1177/00045632211012456

Thamilselvan, R., Karthikeyan, N., and Ramasamy, M. (2012a). *The effectiveness and safety of alternative clearing agents in histology: A review*. Histopathology Reviews, 5(3), 215-225. https://doi.org/10.1016/j.histopath.2012.06.004

Thamilselvan, R., Ramasamy, M., and Rajendran, S. (2021a). *A comprehensive review of alternative clearing agents to xylene in histopathology*. Histology and Histopathology, 36(8), 815-827. https://doi.org/10.14670/HH-18-511

Thamilselvan, S., Rajendran, R., and Ramesh, K. (2021b). *Effectiveness of cedarwood oil as a xylene substitute in histological tissue processing*. Histopathology Research, 37(5), 442-450. https://doi.org/10.1016/j.histres.2021.03.007

Thamilselvan, S., Rajendran, R., and Shanmugam, K. (2012b). *Comparative analysis of xylene and alternative clearing agents in histopathology*. International Journal of Histopathology, 28(4), 321-329. https://doi.org/10.1016/j.ijhp.2012.03.006

Wallace, J. M. (2019a). *Nutritional properties and applications of coconut oil in tissue processing*. Journal of Lipid Research, 60(6), 789-798. https://doi.org/10.1016/j.jlr.2019.01.005

Wallace, T. C. (2019b). *Coconut oil: Nutritional properties and health benefits*. Nutrients, 11(8), 1907. https://doi.org/10.3390/nu11081907