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The Use of Citronella Oil *(Cymbopogon nardus (L.) Rendle)* to Replace Xylene as a Clearing Agent in Tissue Processing

Najwa Syarifah Sadjidah[1], Juliana Christyaningsih[2*], Wisnu Istanto[3]
Department of Medical Laboratory Technology, Poltekkes Kemenkes Surabaya, Indonesia
Corresponding author: juliana_analis@yahoo.co.id

ABSTRACT
Clearing is the process of removing alcohol from the tissue so that paraffin can bind to the tissue. If the tissue still contains alcohol the paraffin cannot bind to the tissue, the tissue will be "ripe on the outside, raw on the inside" and can cause the tissue to be difficult to cut. Xylene is a chemical that is often used in the clearing process, although effective, the use of Xylene has adverse effects on health and is expensive. Therefore, citronella oil is expected to be used as a substitute for Xylene with lower health effects. Citronella oil contains essential oils, the most common compound found in essential oils is terpene. Terpene is a non-polar isolate, non-polar isolates can attract alcohol because alcohol is polar. This study aims to determine whether or not there is a difference in the microscopic picture of preparations that are cleared using Xylene and lemongrass oil in tissue processing. This research was conducted at the Cytohistotechnology Laboratory of the Department of Medical Laboratory Technology of the Poltekkes Kemenkes Surabaya and the Faculty of Veterinary Medicine Airlangga University on January 26-February 25, 2024. The study was processed using kruskal wallis and mann whitney tests with the results of $p = 0.056 > \alpha\ 0.05$, so there were no differences in the quality of hepatic histology preparations of mice. Preparations cleared with Xylene as many as 9 (56.25%) preparations can be said to be good and 7 (43.75%) preparations can be said to be less good. While preparations that are cleared using citronella oil as much as 3 (18.75%) can be said to be good and 13 (81.25%) preparations can be said to be less good. Although there is no difference between the two groups, preparations cleared using Xylene are still better.

Keywords: Clearing, Xylene, Citronella Oil (*Cymbopogon nardus (L.) Rendle*), Microscopic Description of Histological Preparations of Mouse Heparato

INTRODUCTION
Making histology preparations or what is often called tissue processing requires several stages, fixation, dehydration, clearing, infiltration, embedding, and sectioning, then entering the staining process. Fixation is the process of preserving tissue in the laboratory using special fluids, such as formaldehyde, to prevent degradation and maintain the structural integrity of the tissue. Fixation aims to stabilize the tissue in a "life-like state" so that it can be cut into thin sections for microscopic analysis (Bussolati, 2022). The fixation is also often called chemical fixation, a fixation that is often used on preparations observed with a microscope (Musyarifah & Agus, 2018). And Dehydration is removal of water and fixative from tissues using dehydrating fluids (Lerch et al., 2019), reagents that are often used are ethanol and alcohol with graded concentrations of 70%, 80%, 96%, and 100% absolute alcohol (Rahmawanti et al., 2021). Clearing is the replacement of dehydrating fluids with a fluid that is miscible with both the dehydrating fluid and the embedding medium (Chandraker et al., 2019), this step helps to remove any remaining water and is fixative, making the

tissue transparent and ready for embedding.If there is still alcohol in the tissue, the paraffin cannot bind to the tissue, the tissue will be "cooked outside, raw inside" and can cause the tissue to be difficult to cut (Tatyana Gurina & Sims, 2023). To guarantee high-quality results, the processing protocol needs to be adapted to the size and composition of the tissue by modifying variables such as the reagents used and the duration of each step (Aziz & Zeman-Pocrnich, 2022). Infiltration or impregnation is the replacement of the clearing agent with the embedding medium, which is typically paraffin wax. This step ensures that the tissue is fully infiltrated with the wax, allowing it to be cut into thin sections (Dewi et al., 2022). Then sectioning stage, sectioning is cutting the embedded tissue into thin sections (Rahman & Singh, 2024). And last is staining with Hematoxylin-Eosin, staining is applying stains to the sections to enhance contrast and reveal specific cellular components (Javaeed et al., 2021), Hematoxylin is a basic dye that will give a blue color to the cell nucleus, while Eosin is an acidic dye that will give a pink color to the cell cytoplasm and connective tissue (Wahyuni et al., 2020).

Xylene, a chemical often used in the clearing process, has a viscosity of 0.812 Cp and a pH of 6-7. Although effective, the use of xylene has adverse health effects and is expensive. The nervous system, heart, kidneys, and many other health issues can be affected by xylene toxicity. Economic factors are also a concern as the production cost of histology preparations may increase due to the high price of xylene (D'Azzuri, 2023). Xylene is a colorless aromatic hydrocarbon found in liquid or gaseous form naturally in coal, petroleum, and wood. Many histologists prefer xylene because of its ability to remove alcohol from tissues quickly, render them transparent, and aid in paraffin infiltration (Alwahaibi et al., 2020). Cost-effective and environmentally friendly alternatives must be sought. Citronella oil also contains non-polar essential oils, non-polar compounds can attract alcohol, so that paraffin can enter the tissue and can harden (Sari et al., 2022). Citronella oil has a viscosity level that is not much different from xylene, namely 0.9975 cP-1.276 cP and has the advantage of being an environmentally friendly and economical material (Ameliana et al., 2019). In addition, citronella oil has higher economic added value such as citronellal, citronilol, and geraniol (Anwar & Siringoringo, 2020), citronella is usually used as oil for aromatherapy, fragrance and skin care, citronella has purplish stems (Agustina & Jamilah, 2021).

However, not many studies have investigated the use of lemongrass oil as a clearing agent at the tissue processing stage. Therefore, this research aims to study and evaluate the possibility of using lemongrass oil as a clearing agent at the tissue processing stage. Consequently, it is expected that this research will contribute to innovations that will make the histotechnical process more efficient and sustainable. Therefore, the researcher is interested in conducting a study on "the use of citronella oil *(Cymbopogon nardus (L.) Rendle)* to replace xylene as a clearing agent in tissue processing".

RESEARCH METHOD

The type of research used is experimental research. This study aims to determine the effectiveness of citronella oil to replace xylene as a clearing agent in the process of making histology preparations. The variables observed were the clarity of the shape of the cell nucleus and cytoplasm, and color uniformity between blue in the nucleus and red in the cytoplasm when observed microscopically. This study was conducted at the Surabaya Polytechnic Medical Laboratory Technology campus and the Faculty of Veterinary Medicine, Airlangga University in January - March 2024. In this study, there were 2 groups, namely the first group of xylene or control and the second group of citronella oil. The

sample in this study was 16 mice hepatic preparations per group, therefore the number of samples used was 32 mice hepatic preparations. The assessment criteria used are :

Table 1. Histology Preparation Assessment Criteria

No.	Structure	Cell nucleus	Quality	
			Ordinal	Nominal
1.	Cell Nucleus	Blue color and indistinct cell nucleus shape	Not good	1
		Light purplish blue/pale cell nucleus color appears faded and the shape of the cell nucleus is less clear	Not so good	2
		The color of the cell nucleus appears dark purplish blue and appears to contrast with the color of the cytoplasm, and the shape of the cell nucleus is clear.	Good	3
2.	Cytoplasm	Easy red color and indistinct cytoplasm	Not good	1
		The color of the cytoplasm is light pink and appears faded and the cytoplasm is not clear.	Not so good	2
		The color of the cytoplasm is dark pink and strong so that the cytoplasm appears clearer	Good	3
3.	Boundary between cells	Bantas between cells is not clear	Not good	1
		Bantas between cells is not clear	Not so good	2
		Bantas between cells is clear	Good	3
4.	Color uniformity	Non-uniform color	Not good	1
		Less uniform color	Not so good	2
		Uniform color	Good	3

Source (Afrida, 2021)

Table 2. Total Microscopic Assessment Criteria

No.	Description	Value
1.	Not good	1-4
2.	Not so good	5-8
3.	Good	9-12

Source (Afrida, 2021)

The stages carried out include fixation with 10% NBF for 24 hours, dehydration with graded alcohol (70% for 1 hour, 80% for 1 hour, 95% for 2 hours, 95% for 1 hour, 100% for 1 hour, 100% for 1 hour and 100% for 1 hour), clearing (1 hour, 1 hour and 2 hours), impregnation 56 - 58ºC for 2 hours for 3 times (Sudiana, 2023), sectioning, staining using hematoxylin-eosin, and mounting (Maulani, 2020). The tools used in this study were minor surgical equipment (scissors, tweezers, scale, and clamps), Sakura Histo-Tek VP1 tissue processor, cutting board, measuring instrument, screw cap bottle, tweezers, stopwatch, chamber, beaker glass, hot plate, tissue cassette, Sakura Accu-Cut SRM microtome, brush, water bath, object glass, cover glass, and Olympus CX-33 microscope. The materials used in this study were 10% NBF, alcohol (70%, 80%, 90%, 96%, and 100% absolute), paraffin, xylene, citronella oil, hematoxylin-eosin, entelan, and distilled water. The research procedure starts from taking the hepatic organs of mice and then fixated with NBF10% (Setyowati, 2020). After sampling, tissue processing was carried out until the preparation was ready to be read and observed. This research has received Ethical Approval with the number

of No.EA/2079/KEPK-Poltekkes_Sby/2024 by the Health Research Ethics Commission of the Health Research Polytechnic of the Poltekkes Kemenkes Surabaya.

RESULT AND DISCUSSION

This study conducted a clearing process of mice hepatic tissue which was divided into 2 groups, namely group 1 which was cleared using xylene, and group 2 which was cleared using citronella oil *(Cymbopogon Nardus (L.) Rendle)*. In each group, 16 preparations are needed, which means that the total number of preparations used is 32 preparations. In the clearing stage, both groups needed the same time, namely 1 hour for the first soaking, then 1 hour for the third soaking, and 2 hours for the last soaking. The results of clearing using xylene show that of the 16 preparations, 9 (56.25%) preparations can be said to be good while 7 (43.75%) other preparations can be said to be unfavorable. Meanwhile, the results of clearing using citronella oil show that of the 16 preparations, 3 (18.75%) preparations can be said to be good and 13 (81.25%) other preparations can be said to be less good. The assessment is based on the cell nucleus, cytoplasm, intercellular boundaries, and color uniformity read and validated by the doctor.

Table 3. Assessment Results of Preparations Cleared with Xylene and Citronella Oil

Kelompok	Sampel	Inti Sel	Sitoplasma	Batas Antar Sel	Keseragaman Warna	Total
Xylene (A)	A-1	3	3	2	2	10
	A-2	2	2	3	3	10
	A-3	3	2	3	3	11
	A-4	2	3	3	2	10
	A-5	3	3	3	2	11
	A-6	3	2	3	2	10
	A-7	2	1	2	3	8
	A-8	3	3	3	3	12
	A-9	2	1	2	2	7
	A-10	2	1	1	2	6
	A-11	3	1	3	2	9
	A-12	2	2	1	2	7
	A-13	1	1	1	2	5
	A-14	3	3	3	3	12
	A-15	2	3	1	2	8
	A-16	3	3	1	3	10
Average		2.4375	2.125	2.1875	2.375	9.125
Citronella Oil (B)	B-1	1	2	2	1	6
	B-2	2	3	1	2	8
	B-3	2	2	2	2	8
	B-4	2	2	1	2	7
	B-5	2	2	2	2	8
	B-6	2	2	1	3	8
	B-7	3	2	1	1	7
	B-8	3	3	2	2	10
	B-9	1	2	1	2	6
	B-10	2	2	2	2	8
	B-11	3	3	2	2	10
	B-12	2	2	1	2	7
	B-13	2	2	1	2	7
	B-14	3	3	2	2	10
	B-15	2	2	1	3	8

	B-16	2	3	1	2	8
Average		2.125	2.3125	1.4375	2	7.875

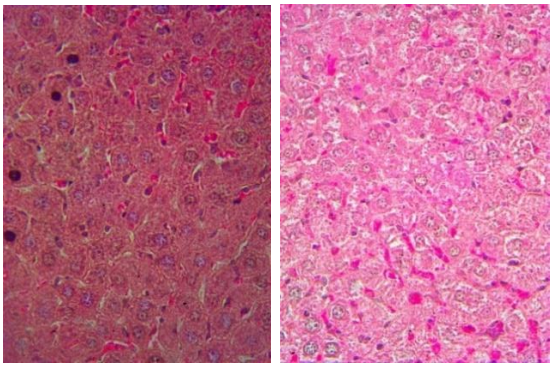

Figure 1. Xylene-cleared preparations (left) with a good category and those cleared with Citronella oil (right) is in the unfavorable category.

Then the research data was tested for normality using Shapiro Wilk, the results of the quality of preparations cleared using xylene had a p-value of 0.310, where this value was greater than the α value (0.05) indicating that the data was normally distributed. Meanwhile, the preparation cleared using citronella oil has a p-value of 0.023, where this value is smaller than the α value (0.05) which indicates that the data is not normally distributed. Then continued the homogeneity test, and obtained a significance value of 0.022, then the value of 0.022 <0.05. So it can be concluded that the two groups of preparations are not homogenized. After obtaining the results of the normality and homogeneity tests, the data was continued with the Kruskal Wallis test and continued with the Mann-Whitney difference test. In both tests, the Sig. (2-tailed) 0.056 where the value is > 0.05 which means H0 is accepted and H1 is rejected. This means that between preparations cleared using xylene and those cleared using citronella oil *(Cymbopogon nardus (L) rendle)*, there is no significant difference.

This study was conducted to determine differences in the quality of mice liver histology preparations cleared using xylene and citronella oil (Cymbopogon Nardus (L.) Rendle). In this study samples of mice hepatic tissue preparations were obtained through the process of surgery to be processed into histology preparations. In each group, there were 16 preparations that were processed differently at the clearing stage, 16 preparations with a clearing process using xylene and 16 preparations cleared using citronella oil (Cymbopogon Nardus (L.) Rendle). The clearing process takes 4 hours, namely, 1 hour, 1 hour, and 2 hours in each treatment group.

Clearing is a process in histology that aims to remove alcohol from the tissue after the dehydration process and replace it with a solution that can bind paraffin. Tissue cannot be put directly into paraffin because alcohol and paraffin cannot bind to each other. Clearing also aims to remove alcohol from the tissue so that it is not susceptible to decay. Good clearing results make the tissue look transparent (Wulandari et al., 2022). Xylene is a clearing agent commonly used in laboratories in the preparation of histology preparations. Xylene gives good preparation results in the clearing stage. However, xylene has a high solubility to dehydrate agents and paraffin and can have a transparent effect on the tissue. However, xylene is not good in terms of safety for laboratory workers because of its hazardous and toxic nature (Selistiyaningsih & Nurhidayati, 2019).

The results of observations on the quality of hepatic histology preparations of mice at the clearing stage with xylene showed a good microscopic picture of 9 out of 16 preparations, while 7 other preparations could be categorized as poor. The results of preparations cleared with citronella oil show a good microscopic picture of as many as 3 out of 16 preparations. While 13 other preparations can be categorized as poor. From the results of the research that has been done, there is no difference in the quality of hepatic histology preparations of mice cleared with

xylene and cleared with citronella oil *(Cymbopogon Nardus (L.) Rendle)* at the tissue processing stage. The results of the Kruskal Wallis and Mann Whitney statistical tests showed p = 0.056, where the results were p> α (0.05). The conclusion H0 is accepted, which means that there is no difference in the quality of preparations cleared using xylene and those cleared using citronella oil *(Cymbopogon Nardus (L.) Rendle)* at the tissue processing stage. This is because citronella oil contains essential oils, most of which consist of terpenes, terpenoids, aromatic components, and other aliphatics and are characterized by low molecular weight. Essential oils are composed of a complex mixture of volatile to semi-volatile, polar, and non-polar compounds. Terpenes are the most common type of compounds found in essential oils. Terpenes are synthesized through the mevalonate pathway in the cytoplasm of plant cells (Butnariu & Sarac, 2018). Terpenes are non-polar isolates, non-polar isolates can attract alcohol because alcohol is polar (Miastkowska & Śliwa, 2020).

CONCLUSION AND RECOMMENDATION

The clearing process using xylene and citronella oil *(Cymbopogon Nardus (L.) Rendle)* has no difference, therefore citronella oil *(Cymbopogon Nardus (L.) Rendle)* is expected to be used to replace xylene as a clearing agent in tissue processing. Although there is no difference between the two groups, preparations cleared using xylene are still better. The use of citronella oil as a substitute for xylene as a clearing agent produces a fairly good preparation quality almost the same as clearing using xylene.

REFERENCES

1. Ahmed, A., Morley, E., Hameed, F. A., Afrida, A. D. (2021). Gambaran Histologi Jaringan Hepar Mencit (Mus Musculus) Yang Difiksasi Dengan Larutan Carnoy Dengan Variasi Waktu 4 Jam, 8 Jam Dan 12 Jam. Https://Repository.Poltekkes-Smg.Ac.Id/Index.Php?P=Show_Detail&Id=24382&Keywords=
2. Agustina, A., & Jamilah, M. (2021). Kajian Kualitas Minyak Serai Wangi (Cymbopogon Winterianus Jowitt.) Pada Cv Ab Dan Pt. Xyz Jawa Barat. Agro Bali: Agricultural Journal, 4(1), 63–71. Https://Doi.Org/10.37637/Ab.V4i1.681
3. Alwahaibi, N., Aljaradi, S., & Alazri, H. (2020). Alternative To Xylene As A Clearing Agent In Histopathology. 189–193. Https://Doi.Org/10.4103/Jlp.Jlp
4. Ameliana, L., Almawadah, A., & Wulandari, L. (2019). The Effect Of Citronella Oil Concentration (Cymbopogon Nardus (L.) Rendle) On The Quality Of Shampoo And Antifungal Activity Of Candida Albican. Indonesian Journal Of Pharmaceutics, 1(2), 52–56. Https://Jurnal.Unpad.Ac.Id/Idjp/Article/View/21551/Html_1
5. Anwar, Y., & Siringoringo, V. S. (2020). Fractionation Of Citronella Oil And Identification Of Compounds By Gas Chromatography-Mass Spectrometry. Pharmaceutical Sciences And Research, 7(3), 138–144. Https://Doi.Org/10.7454/Psr.V7i3.1101
6. Aziz, S. J., & Zeman-Pocrnich, C. E. (2022). Tissue Processing. National Library Of Medicane, 47–63. Https://Pubmed.Ncbi.Nlm.Nih.Gov/34859398/
7. Bussolati, G. (2022). Fixation In Histopathology: The Mandate To Renew. Pathologica, 114(4), 275–277. Https://Doi.Org/Https://Doi.Org/10.32074%2f1591-951x-782
8. Butnariu, M., & Sarac, I. (2018). Essential Oils From Plants. Journal Of Biotechnology And Biomedical Science, 1(4). Https://Doi.Org/Https://Openaccesspub

.Org/Jbbs/Article/940
9. Chandraker, R., Rathod, V. C., Chandraker, N. K., Pundir, S., Dixit, S., & Desai, V. (2019). No Titlecomparison Between Xylene And Coconut Oil In Tissuee Processing. Modern Medical Laboratory, 1(2), 96–99. Https://Doi.Org/Comparison Between Xylene And Coconut Oil In Tissuee Processing
10. D'azzuri, B. M. (2023). Vegetable Oils As A Substitute Of Toxic Chemicals In The Histopathological Clearing Process. Medula (Medical Professsion Journal Of Lampung), 13. Https://Doi.Org/10.53089/Medula.V13i6.813
11. Dewi, H., Quzwain, F., & Wulansari, N. (2022). Histology Slide Quality Comparative Study; Impregnation And Embedding Using Beeswax And Paraffin. Jambi Medical Journal, 10(2). Https://Online-Journal.Unja.Ac.Id/Kedokteran/Article/View/19242
12. Javaeed, A., Qamar, S., Ali, S., Mustafa, M. A. T., Nusrat, A., & Ghauri, S. K. (2021). Histological Stains In The Past, Present, And Future. Cureus, 13(10). Https://Doi.Org/Https://Doi.Org/10.7759%2fcureus.18486
13. Lerch, M. L., Baurer, D. R., Theiss, A., Chafin, D., Otter, M., & Baird, G. S. (2019). Monitoring Dehydration And Clearing In Tissue Processing For High-Quality Clinical Pathology. Biopreservation And Biobanking, 17(4), 303–311. Https://Doi.Org/Https://Doi.Org/10.1089%2fbio.2018.0122
14. Maulani, Y. (2020). Gambaran Makroskopis Jaringan Hepar Tikus (Rattus Norvegicus) Yang Diproses Menggunakan Minyak Cengkeh (Syzigium Aromaticum) Sebagai Alternatif Larutan Clearing. Https://Librepo.Stikesnas.Ac.Id/222/
15. Miastkowska, M., & Śliwa, P. (2020). Influence Of Terpene Type On The Release From An O/W Nanoemulsion: Experimental And Theoretical Studies. Pubmed Central, 25(12). Https://Doi.Org/Https://Doi.Org/10.3390%2fmolecules25122747
16. Musyarifah, Z., & Agus, S. (2018). Tinjauan Pustaka Proses Fiksasi Pada Pemeriksaan Histopatologik. 7(3), 443–453. Http://Jurnal.Fk.Unand.Ac.Id/Index.Php/Jka/Article/View/900
17. Rahman, H. A., & Singh, A. P. (2024). Section Cutting In Histopathology : An Update. April.
18. Rahmawanti, A., Setyowati, D. N., & Mukhlis, A. (2021). Histopathological Of Brain, Eye, Liver, Spleen Organs Of Grouper Suspected Vnn In Penyambuan Village, North Lombok. Jurnal Biologi Tropis, 21(1), 140–148. Https://Doi.Org/10.29303/Jbt.V21i1.2439
19. Sari, I., Faradilla, M., & Ayuningsih, C. M. (2022). Antibacterial Activity Of Citronella Essential Oil From Cymbopogon Nardus (L .) Rendle) Against Methicillin- Resistant Staphylococcus Aureus. 05(1), 16–22. Https://Talenta.Usu.Ac.Id/Idjpcr/Article/Download/8302/5482
20. Selistiyaningsih, S. R., & Nurhidayati, T. (2019). Tantangan Implementasi Hasil Riset Perguruan Tinggi Untuk Industrialisasi. Penilaian Diri Tentang Kompetensi Psikomotor Komunikasi Terapeutik Mahasiswa Profesi Ners Universitas Muhammadiyah Semarang, 2, 224–232. Https://Prosiding.Unimus.Ac.Id/Index.Php/Mahasiswa/Article/View/465
21. Setyowati, E. D. (2020). Gambaran Mikroskopis Sediaan Jaringan Hepar Mencit (Mus Musculus) Yang Dijernihkan (Clearing) Menggunakan Cedar Wood Oil (Cedrus Sp) Dengan Pewarnaan Hematoksilin Eosin. 1–3. Https://Repository.Poltekkes-Smg.Ac.Id/Reader/Index.Html?Token=A4b33244c155a17170a3bf8f0b367fddfcf95e3b3ae72a90c6d0d6aec027bda6

&Fid=62427&Bid=22207
22. Sudiana, I. K. (2023). Sitohistoteknologi.
23. Tatyana Gurina, S., & Sims, L. (2023). Histology, Staining. National Library Of Medicane. Https://Www.Ncbi.Nlm.Nih.Gov/Books/Nbk557663/
24. Wahyuni, F. I., Septiadi, M. G. S., Pitriani, & H. Susanto, C. (2020). Verifikasi Metode : Analisa Pewarnaan Umum Histopatologi Hematoxylin Dan Eosin Modifikasi Untuk Negri Bodies Rabies. Repository Pertanian, Halaman 67-74. Https://Repository.Pertanian.Go.Id/Bitstreams/607bde47-036d-4384-80b4-Fa91f2b7f11a/Download
25. Wulandari, Y. P., Nuroini, F., & Ariyadi, T. (2022). Gambaran Jaringan Hati Pada Tahap Clearing Menggunakan Ekstrak Jeruk Nipis Pada Pewarnaan Hematoksilin Eosin. Biology Natural Resource Journal (Binar), 1(1), 44–47. Http://Journal.Unirow.Ac.Id/Index.Php/Binar/Index