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Evaluating the Efficacy of Lemongrass (*Cymbopogon Citratus)* Oil as a Safer Alternative to Xylene in Tissue Processing: An Experimental Study

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KEYWORDS

Xylene, Clearing agent, Histopathology, Tissue processing

ABSTRACT:

Introduction: The organic solvent xylene has traditionally been used as a clearing agent in tissue processing. Since xylene is known for its toxicity and carcinogenicity, other safer organic solvents, such as vegetable oils and mineral oils, have been tried by various researchers in the past. So, in this study, to overcome the toxic effects of xylene and to replace it with a safe, non-toxic, non-carcinogenic, environment-friendly alternative, an essential oil such as lemongrass (*Cymbopogon citratus)* oil was evaluated.

Methods: A total sample size of 60 tissue specimens from the head and neck region was included in the study. From each of these specimens, two tissue bits were cut and subjected to parallel processing in xylene and an essential oil, such as lemongrass (*Cymbopogon citratus)* oil. The specimens cleared in xylene and specimens cleared in lemongrass oil were examined for multiple parameters, including tissue rigidity, translucency, shrinkage, completeness of impregnation, microtomy, staining quality, and microscopic clarity and a comparison was made between the two groups.

Statistical analysis: The obtained data was subjected and recorded as frequency and percentage categorical values, and the Shapiro-Wilk test and Wilcoxon test were used for comparing two groups

Results: Most of the parameters, such as rigidity of specimen after clearing, translucency, completeness of impregnation, and staining quality, were found to be equivalent for lemongrass oil compared to that of xylene. However, significantly less shrinkage of the specimen after clearing was evident in specimens treated with this oil compared to xylene, likely due to their lower volatility and weaker fat-solvent properties. Lemongrass oil performed best, offering consistent clearing effects, good translucency, and satisfactory staining.

Conclusions: The findings indicate that lemongrass oil may be a promising and safe alternative to xylene as a clearing agent in tissue processing, showing optimal clearing properties, tissue clarity and staining results.

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1. Introduction

Tissue processing in histopathology involves many procedures, and clearing is one of the most important steps for light microscopy. The aromatic hydrocarbon, xylene (also known as dimethylbenzene [C_6H_4 $(CH_3)_2$]), is the most commonly used clearing agent. Xylene is widely preferred by many of the histologists as it rapidly removes alcohols from tissues, makes them transparent, and facilitates paraffin infiltration [1]. The main issue with using xylene is its toxicity; long-term exposure is especially harmful to health. Contact with xylene occurs through the breath, eye, oral, and dermal routes [1]. The various toxic effects of xylene include respiratory difficulties, breathlessness, various gastrointestinal complications like loss of appetite, vomiting, other physical issues like fatigue and dizziness, impaired neural coordination, memory impairment, loss of coordination and judgement, coma and potentially fatal outcomes [2,3,4]. It can also lead to hepatotoxicity and renal toxicity [5]. Additionally, it is a proven carcinogen. Considering xylene's toxic potential, the discovery of bio-friendly alternatives to it is the present need. In pursuit of finding such a safe substitute, various studies have been done in the past with limonene reagents, aliphatic hydrocarbons, vegetable oils and mineral oils [6,7,8,9,10,11,12,13]. However, these studies were found to be less effective.

Lemongrass (*Cymbopogon citratus*) oil is an essential oil, that has a variety of health benefits. It is well-known for its medicinal properties and is commonly found in traditional medicine, pharmaceuticals, perfumery, and cosmetics. It is readily available in the market. It is a non-toxic and bio-friendly oil with added therapeutic benefits, making it efficient for treating coughs, influenza, headaches, gingivitis, pneumonias, and vascular disorders. Its cleansing properties detoxify the liver, kidneys, bladder, pancreas, and digestive tract. It contributes to lowering cholesterol, excess fats, uric acid, and various toxins in the body. Additionally, it supports digestion, promotes lactation, enhances blood circulation, and helps treat gastroenteritis and indigestion [14]. In the present study, we assessed the effectiveness of lemongrass (*Cymbopogon citratus)* oil as a clearing agent in histopathology by comparing it with xylene.

2. Methods

The study involved a sample size of 60 tissue specimens, which were organised into two groups of 30 samples each, sourced from the archives of the Department of Oral Pathology and Microbiology at Yenepoya Dental College after obtaining the institutional ethics committee approval (protocol number: YEC-1/2023/078). Two tissue bits were cut from each specimen and subjected to parallel processing in xylene and lemongrass (*Cymbopogon citratus)* oil. Two separate processing stations for manual tissue processing were arranged; one with regular reagents used for routine processing, i.e. ascending grades of alcohol for dehydration and two changes of xylene for clearing and paraffin wax for impregnation. In the second station, the clearing agent, xylene, was replaced with two changes of lemongrass (*Cymbopogon citratus)* oil (Figure 1 and 2).

Figure 1: Processing station containing xylene as clearing agent

Figure 2: Processing station containing lemongrass oil as clearing agent

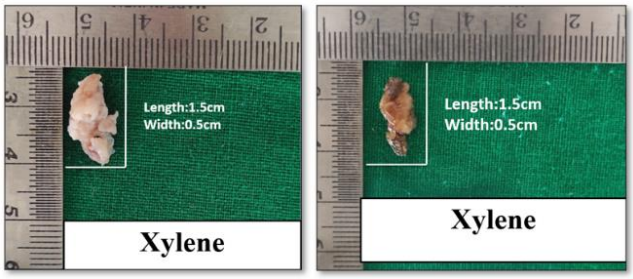

Figure 3: Measurement of tissue before and after clearing in xylene

Journal of Chemical Health Risks
www.jchr.org
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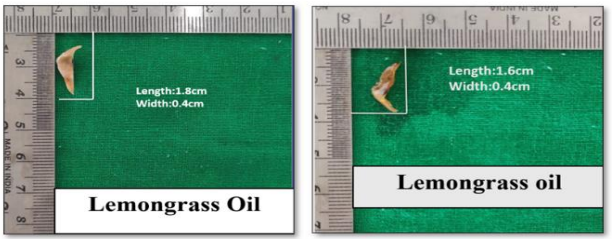

Figure 4: Measurement of tissue before and after clearing in lemongrass oil

The tissue specimens processed in two different stations were analysed for different parameters such as rigidity and translucency of the tissue after clearing, shrinkage of the tissue after clearing, completeness of paraffin wax infiltration in cleared tissues, microtomy parameters such as ease of sectioning, ribbon formation and sectioning artefacts (Figure 3 and 4). All the sections obtained were stained by H&E staining, and additionally, evaluation of the staining quality of cleared specimens and microscopic clarity was also done (Figure 5 and 6). All the findings comparable to the control (xylene) specimens were graded as score 1, less than control as score 0 and better or more than control as score 2.

All these parameters were checked and recorded by two independent observers and twice on different occasions by each observer, in order to remove the inter- and intra-observer bias. The obtained data was subjected and recorded as frequency and percentage for categorical values and the Shapiro-Wilk test and Wilcoxon test were used for comparing two groups.

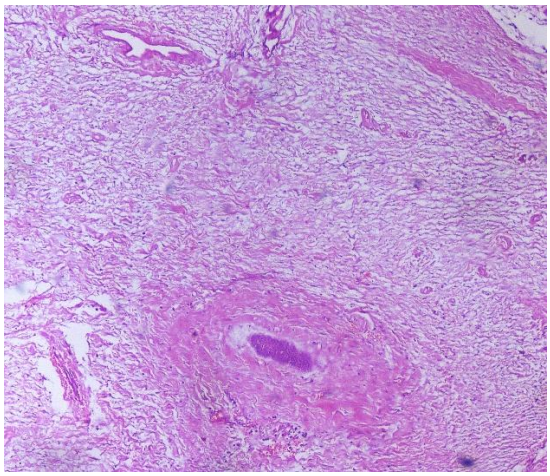

Figure 5: Photomicrograph of tissue cleared in xylene

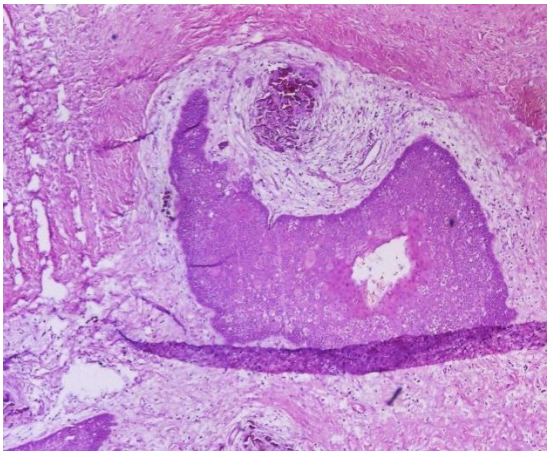

Figure 6: Photomicrograph of tissue cleared in lemongrass oil

3. Results

In this study, when specimens cleared in lemongrass oil and xylene (control) were compared, lemongrass oil showed equal rigidity (100% for observer 1 and 93.3% for observer 2), equal translucency (86.6% for observer 1 and 100% for observer 2) and equal completeness of impregnation (80% for observer 1 and 90% for observer 2) to that of control. Less shrinkage was observed by lemongrass oil when compared to the control (100% consistency for both observers).

Lemongrass oil				
Parameters		**Score 0**	**Score 1**	**Score 2**
1.Rigidity	Observer 1	0	30 (100%)	0
	Observer 2	2 (6.7%)	28(93.3%)	0
2.Translucency	Observer 1	4(13.3%)	26 (86.66%)	0
	Observer 2	0	30(100%)	0
3.Shrinkage	Observer 1	30(100%)	0	0
	Observer 2	30(100%)	0	0
4.Completeness of impregnation	Observer 1	6 (20%)	24 (28%)	0
	Observer 2	3 (10%)	27 (90%)	0

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5.Ease of sectioning	Observer 1	2 (6.7%)	26 (86.6%)	2 (6.7%)
	Observer 2	4 (13.4%)	26 (86.6%)	0
6. Ribbon formation	Observer 1	5 (16.7%)	21 (70%)	4(13.3%)
	Observer 2	13 (43.3%)	14 (46.7%)	3(10%)
7. Sectioning Artefacts	Observer 1	2(6.7%)	22(73.3%)	6(20%)
	Observer 2	4(13.3%)	22(73.3%)	2(6.7%)
8.Staining Quality	Observer 1	3 (10%)	21(70%)	6(20%)
	Observer 2	3 (10%)	18(60%)	9 (30%)
9.Clarity of cellular details	Observer 1	11(36.7%)	19(63.3%)	0
	Observer 2	5(16.7%)	25(83.3%)	0
10.Clarity of nuclear details	Observer 1	17(56.7%)	13(43.3%)	0
	Observer 2	9(30%)	21(70%%)	0

Table 1: Observations obtained by comparing lemongrass oil and xylene, recorded as frequency and percentage.

When microtomy parameters were considered, lemongrass showed comparable ease of sectioning (86.6% for both observer 1 and observer 2) and comparable ribbon formation (observer 1 reporting 70% and observer 2 reporting 46.7%). Lemongrass oil showed the highest proportion of comparable artefacts to that of the control (73.3% for observer 1 and observer 2). Fewer artefacts than the control were noted at 20% and 6.7% by observer 1 and observer 2, respectively and more artefacts at 6.7% and 13.3 % by observer 1 and observer 2, respectively.

Lemongrass oil-treated tissues also had predominantly satisfactory quality of staining (70% for observer 1 and 60% for observer 2). Good quality of staining was noted at 20% and 30% by observers 1 & 2, respectively. Lemongrass oil-treated tissues had the highest proportion of distinct architecture and nuclear-cytoplasmic contrast with that of the control (63.3% for observer 1 and 83.3% for observer).

It also showed a higher proportion of distinct chromatin condensation, prominent nuclear membrane and crisp staining of the nucleus with that of the control (43.3% by observer 1 and 70% by observer 2), while indistinct smudging and pyknosis of the nuclei was seen in 56.7% (observer 1) and 30% (observer 2) respectively. (Table 1)

Shrinkage							
Parameters			**Mean**	**SD**	**Median**	**IQR**	**p value (paired t test[a] or Wilcoxon test [b])**
Xylene (mm)	Length	Before	1.80	0.72	1.45	0.9	<0.001[b]
		After	1.74	0.71	1.40	0.8	
	Width	Before	0.72	0.20	0.70	0.3	<0.001[a]
		After	0.63	0.20	0.70	0.3	
Lemongrass oil(mm)	Length	Before	1.65	0.64	1.50	0.8	<0.001[b]
		After	1.57	0.66	1.50	0.7	
	Width	Before	0.74	0.27	0.70	0.5	<0.001[b]
		After	0.69	0.26	0.60	0.4	

Table 2: Measurement of tissue bits taken before and after processing to compare the gross- shrinkage

Statistical analyses were carried out using IBM SPSS 27 software. The quantitative variables under study were presented using mean (Standard Deviation) and Median (Inter Quartile Range). Further, the data was checked for Normality using the Shapiro-Wilk test. A paired t-test was used to compare the mean of two dependent variables if those variables follow a normal distribution. The Wilcoxon test is used to compare the means of two dependent variables if those variables do not follow a normal distribution. A p-value ≤ 0.05 was considered statistically significant. (Table 2)

4. Discussion

Clearing agents serve a crucial role in tissue processing as intermediate solvents are fully miscible with both ethanol and paraffin wax. Xylene, while widely used for its effectiveness, is a known carcinogen and poses significant health and environmental risk, necessitating the exploration of safer substitutes. Several substitutes, including mineral and vegetable oils, have been explored as alternatives to xylene due to its toxicity

Journal of Chemical Health Risks
www.jchr.org
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[6,7,8,15,16,17,18,19]. This comprehensive study assessed the effectiveness of an essential oil, lemongrass (*Cymbopogon citratus)* oil, as a clearing agent to replace the toxic xylene from the histopathology laboratory. This oil was chosen for this study based on the fundamental characteristic that oils can mix with both the dehydrating agent alcohol and the molten embedding medium paraffin.

This study considered and compared multiple parameters, including tissue rigidity, translucency, shrinkage, impregnation, microtomy performance, staining quality, and clarity of cellular and nuclear details in order to find a safer and bio-friendly alternative to xylene.

When the rigidity was compared, lemongrass oil-treated tissues showed equal rigidity compared to that of the control (xylene). This may be due to the presence of citral, which is a primary compound in lemongrass oil which gives a more consistent clearing effect due to its ability to penetrate tissue and promote uniform tissue interaction, potentially softening collagen fibers more effectively [14].

When translucency and comparable completeness of impregnation were compared, lemongrass oil-treated tissues showed equal translucency and equal completeness of impregnation, compared to that of the control (xylene). It is well understood that the translucency of the specimen observed after clearing is because of the high refractive index of xylene (1.495), closer to that of tissue proteins (1.33-1.4). From this finding, we also like to state that the organic oil used in this study is miscible with paraffin and does not interfere with the impregnation of the molten paraffin into the tissue.

In this study, we observed that the shrinkage of the tissues was less compared to xylene. Some degree of shrinkage of the tissue is reported after clearing in xylene and is considered to be due to the extraction of fat by xylene. Xylene is a fat solvent and therefore removes the fat from the tissue, resulting in shrinkage of the tissue. In addition, evaporation of some amount of highly volatile xylene trapped in tissue spaces also could have contributed to the volume change noted. On the contrary, the oil used in the study is neither a good fat solvent, nor volatile compared to xylene and therefore less volume change or shrinkage. Our findings are comparable to the observations of Sermadi et al., who used coconut oil as a clearing agent and reported a reduced shrinkage compared to xylene-treated specimens. Morphometric analysis done by the same researchers found a significant reduction in the mean cell area in xylene-treated specimens compared to that in coconut oil-treated specimens endorsing the reason for shrinkage [7]. Lemongrass oil group scored readings comparable to xylene group in terms of ease of sectioning and ribbon formation. This can be attributed to its higher viscosity. Similar artefacts were seen with the lemongrass oil cleared specimens compared to those of the xylene group of specimens. In contrast to our observation, Udonkang et al. noted only minor differences in the sectioning of tissues processed with bleached palm oil at 60 °C as an alternative for xylene in clearing as well as deparaffinization procedures [8]. Lemongrass oil, which has a relatively lower viscosity and smaller molecular size (due to its high concentration of volatile compounds like citral), can penetrate tissues more effectively without causing them to become too soft, which helps in smooth and clean sectioning.

When the quality of staining was studied, lemongrass oil was scored satisfactorily. When clarity of cellular details and nuclear details were graded, it showed distinct architecture and good nuclear-cytoplasmic contrast as well as distinct chromatin condensation, prominent nuclear membrane and crisp staining. None of the observers showed a superior clarity of cellular details. Madhura MG et al. demonstrated a finding when bleached palm oil and xylene were used. The study demonstrated slightly superior clarity and staining results, with 100% adequacy compared to 91.6-91.7% for bleached palm oil [10].

Thus, we concluded that lemongrass oil may probably be substituted for xylene without losing valuable diagnostic information in the histopathology laboratory.

5. Conclusion

Lemongrass oil emerged as a favourable substitute due to its superior consistency across various parameters. It demonstrated excellent translucency, minimal tissue shrinkage, and comparable rigidity to xylene, ensuring

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effective tissue preparation for subsequent processing. Completeness of impregnation was also notable, with lemongrass oil achieving results close to xylene, facilitating uniform paraffin wax infiltration. Microtomy results were similarly promising, as tissues processed with lemongrass oil displayed ease of sectioning, consistent ribbon formation, and minimal artefacts. The clarity of cellular and nuclear details was largely preserved, indicating that lemongrass oil does not interfere with critical diagnostic features. Thus, the study highlighted the feasibility of integrating essential oil, particularly lemongrass oil, into the histopathology workflow as a safer alternative to xylene.

References

1. Niaz K, Bahadar H, Maqbool F, Abdollahi M. A review of environmental and occupational exposure to xylene and its health concerns. EXCLI journal. 2015;14:1167.
2. Nylen P, Ebendal T, Eriksdotter-Nilsson M, Hansson T, Henschen A, Johnson AC. Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to xylene. Arch Toxicll 1989;63:296-307.
3. Uchida Y, Nakatsuka H, Ukai H, Watanabe T, Liu YT, Huang MY. Symptoms and signs in workers exposed predominantly to xylene. Int Arch Occup Environ Health 1993;64:597-605.
4. Savoleinen H, Pfaffli P, Dose dependant neurochemical changes during short term inhalation exposure to xylene. Arch Toxicol 1980;45:117-22.
5. Kandyala R, Raghavendra SP and Rajasekharan ST. Xylene: An overview of its health hazards and preventive measures. J Oral Max Pathol 2010;14:1-5
6. Swamy SRG, Nandan SRK, Kulkarni PG, Rao TM, Palakurthy P. Bio-Friendly Alternatives for Xylene – Carrot oil, Olive oil, Pine oil, Rose oil. *Journal of Clinical and Diagnostic Research.* 2015;9(11):16–18.
7. Sermadi W, Prabhu S, Acharya S, Javali SB. Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory. Journal of Oral and Maxillofacial Pathology. 2014 Sep 1;18(Suppl 1):S49-53.
8. Udonkang M, Eluwa M, Ekanem TB, Asuquo OR, Akpantah AO. Bleached palm oil as substitute for xylene in histology. *Journal of pharmacy and clinical sciences.* 2014;8(1):8–17.
9. Buesa RJ. Mineral oil: The best xylene substitute for tissue processing yet. J Histotechnol 2000;23:143-9.
10. Madhura MG, Bhavana VS, KumarBV, Suma S, Sarita Y. Bleached vegetable oil as a suitable bio-safe alternative to xylene: An exploratory study. J Adv Clin Res Insights 2016;3:185-189.
11. Rasmussen B, Hjort K, Mellerup I, Sether G and Christensen N. Vegetable oils instead of xylene in tissue processing. Acta Pathol Microbio Immunol Scandinavica 1992;100:827-31
12. Andre GG, Wenger JB, Rebolloso D, Arrington JB, Mehm WJ. Evaluation of clearing and infiltration mixtures (CIMs) as xylene substitutes for tissue processing. J Histotechnol 1994;17:137-42.
13. Lyon H, Holm I, Prento P, Balslev E. Non-hazardous organic solvents in the paraffin embedding technique: A rational approach. Histochem Cell Biol 1995;103:263-9.
14. Nayik GA, Gull A, editors. Antioxidants in Vegetables and Nuts-Properties and Health Benefits. Singapore:: Springer; 2020 Dec 1.
15. Whalen JD, Dufrense RG Jr, Wilkel CS. Xylene substitutes in frozen sections. Dermatol Surg 1995;21:241-2.
16. Ghosh S, Rao RS, Nambiar S, Haragannavar VC, Augustine D, Sowmya SV, Patil S. Quest for biofriendly Xylene substitutes in histopathology: a comparative study. Journal of International Oral Health. 2016 Dec 1;8(12):1101.
17. Bordoloi B, Jaiswal R, Tandon A, Jayaswal A, Srivastava A, Gogoi N. Evaluation and comparison of the efficacy of coconut oil as a clearing agent with xylene. Journal of Oral and Maxillofacial Pathology. 2022 Jan 1;26(1):72-6.
18. Falkeholm L, Grant CA, Magnusson A, Möller E. Xylene-free method for histological preparation: A multicentre evaluation. Lab Invest 2001;81:1213-21.
19. Ankle MR, Joshi PS. A study to evaluate the efficacy of xylene-free hematoxylin and eosin staining procedure as compared to the conventional hematoxylin and eosin staining: An experimental study. J Oral Max Pathol 2011;15:161-7.