



Cedarwood Oil as a Substitute for Xylene in Histopathological Laboratories – A Systematic Review

R. Deepa Viswasini^{1*}, Pratibha Ramani¹ and Abhilasha Ramasubramanian¹

¹*Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamil Nadu, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Xylene is an aromatic hydrocarbon that's extensively used as a deparaffinizing agent, and it's far extraordinarily biohazardous. It is proven to be the best chemical to be used in histopathological laboratories in spite of its carcinogenic potential.

Aim: The aim of this study is to do a systematic review on the efficacy of cedarwood oil as clearing agent and deparaffinizing agent alternative to xylene in histopathological laboratories.

Materials and Methods: The articles for this review were searched from PubMed, Google Scholar, Google search and manual search using the keywords cedarwood oil, natural substitute, natural alternative and xylene. The final of 3 articles were included in the review which compared the efficacy of cedarwood oil as a natural alternative with the gold standard xylene. Once the articles to be reviewed were finalized, data was collected, tabulated and was verified and interpreted.

Results: Three articles included in this review showed considerable and acceptable results with cedarwood oil as clearing and deparaffinizing agent.

Conclusion: Merits and demerits are equally found in both the reagents but compromising the time and cost in terms of cedarwood oil for health benefits hold good for the pathologists but bad for the patients. In case of xylene, compromising the health benefits of workers which results in quicker diagnosis hold good for the patients but not for the pathologists. Finally storage and disposal of these two reagents still remains questionable.

Keywords: Cedarwood oil; substitute; xylene.

1. INTRODUCTION

Cedarwood oil is extracted from a tree, Juniperus and cypresses with cedrol, cedrene, cedrenol, cedral, cuperene, thujopsene, and widdrol as its primary components [1]. It has been found that this oil is known to have several medicinal properties such as antibacterial, antifungal, antispasmodic, antiseptic and also insecticidal [2]. It is also characteristic for its aromatic properties, which made it an important component in cosmetic industry. It has also been beneficial for skin because of its clearing properties [2]. This concept was utilized by the laboratories to be used as clearing agent in histopathological tissue processing and deparaffinizing agent. Histopathological laboratories function in an ambience surrounded by multiple chemical compounds for fixation of tissue specimen, tissue processing, decalcification, staining, etc. which are highly toxic and hazardous to the pathologists such well as the technicians. These chemical compounds cause debilitating effects on skin, eyes, nervous system, blood, liver and kidneys [3]. So a natural alternative is always encouraged. But this replacement of gold standard chemicals with a substitute should not interfere with the diagnostics.

Cedarwood oil to be used as an alternative clearing or deparaffinizing agent to xylene is a challenge. Xylene is an integral component in histopathological laboratories for the last six decades with its efficacy not matched by any other substitutes. Yet, it is the most volatile compound of all other chemical reagents manifesting skin reactions, cardiac and blood ailments, neural and renal toxicities with its inflammable properties [4]. The tissue specimens subjected to xylene leads to structural alterations to an acceptable level. Also disposal of this volatile compound is always a problem. On comparison, Cedarwood oil is a safe, natural alternative, eco-friendly, nonflammable, effortlessly available, and easy to deal with and especially its non-carcinogenic [5]. The major advantage of all is that cedarwood oil does not cause any damage to the tissues processed. But it takes a longer time than xylene to process the tissues and this could vary with the viscosity of the oil used. The viscosity would interfere with the penetration of the oil into the tissues and finally influencing on its onset and duration of action to justify its purpose [6]. Also over a period

of time, crystals are noted which eliminates the question of storage and reusability. So cedarwood oil as well as xylene has both merits and demerits. The present study aims on performing a systematic review to evaluate the efficacy of cedarwood oil as clearing and deparaffinizing agent in histopathological laboratories.

2. MATERIALS AND METHODS

2.1 Search Strategy for Identification of Studies

The search was conducted in accordance with the Cochrane guidelines for systematic reviews. Papers were gathered from PubMed, Google Scholar, Google search and manual search. We also used the internet to identify articles pertaining to our areas of interest. This study comprised of articles that evaluated efficacy of cedarwood oil in histopathological laboratories as an alternative to xylene. The articles were extracted and evaluated further.

2.2 Search Methodology

The search methodology applied was using the following keywords:

“Cedarwood oil” AND “Natural alternative” OR “Natural substitute” AND “Xylene”

To ensure that no relevant publications were ignored, the systematic search was confined to these two terms. Adding another term would have restricted the search, but it would have also increased the chances of missing significant titles. Following the removal of duplicates, titles were reviewed separately by two researchers using established inclusion and exclusion criteria. The remaining papers were thoroughly examined, and a decision was reached based on the relevance of the abstracts and full contents.

2.3 Inclusion Criteria

- Original research articles done with cedarwood oil as clearing agent were included.
- Original research articles done with cedarwood oil as deparaffinizing agent were included.
- Articles published in English language were included in the review.

2.4 Exclusion Criteria

- Studies with no control group and review articles were excluded.
- Studies published in other languages were excluded.
- Studies that used natural essential oils other than cedarwood oil were excluded.
- Studies conducted with different applications of cedarwood oil other than clearing and deparaffinizing agent were excluded from the review.

2.5 Data Extraction

Once the articles to be reviewed were finalized, data was collected from each article, tabulated and was verified and interpreted.

3. RESULTS

3.1 Study Selection

The initial search yielded 76 results. 73 articles were excluded based on the exclusion criteria, title and abstract screening reviews. 2 articles were approved for full text review from Google scholar and an additional of 1 article was included from Google search, manual search & cross references. After the final full text review, 3 articles were included in this systematic review. Data was extracted from the full text articles and reviewed and extracted content. The Fig. 1 presents the search flowchart.

3.2 Study Characteristics

The study characteristics of the articles included in this review are summarized in Table 1. The studies included in this review have evaluated the efficacy of cedarwood oil as clearing or deparaffinizing agent in alternative to xylene.

Two studies have performed with cedarwood oil as a deparaffinizing agent whereas one study has used the same as clearing agent. The evaluation of its efficacy is determined with the parameters analyzed and the scoring criteria given for the analyzed parameters. All the studies have used different parameters with different scoring criteria.

One study has used cedarwood oil as deparaffinizing agent whose evaluation was based on the 2 major parameters, cellular architecture and staining quality. The scoring criteria used for Cellular architecture were 1)

SCORE 0: Indistinct nucleus – cytoplasm; 2) SCORE 1: Distinct Nucleus – cytoplasm and Quality of staining were 1) SCORE - 0 = Poor; 2) SCORE - 1 = Satisfactory 3; 3) SCORE - 2 = Good. They found to obtain good results but couldn't match up to the level of xylene [7].

Another study which used cedarwood oil as deparaffinizing agent evaluated nuclear and cytoplasmic details; Clarity and Uniformity of staining with adequate (score=1) and inadequate (score=0) scores. This study was able to produce good quality in staining with sufficient clarity and uniformity of staining [8].

Cedarwood oil was used as a clearing agent in one study. The parameters evaluated with the respective scores are as follows. Nuclear and cytoplasmic staining: (1) Poor Staining with poor morphology, (2) fair staining, (3) good staining and (4) excellent staining; Background staining: (0) Nil, (1) mild, (2) moderate and (3) sever background staining and artifacts: Presence/absence of artifacts. This study showed results better than the gold standard xylene and has suggested standardizing further [9].

4. DISCUSSION

Two studies have used cedarwood oil as deparaffinizing agent and have proved to show acceptable results. K.Sithara et al. [7] has found results better in terms of cellular architecture more than the staining quality. This study did not positively conclude that cedarwood oil can be used as a definitive alternative to xylene. In contrast Indu S et al. [8] has utilized 8% cedarwood oil and found that it has produced desirable and acceptable results in terms of Nuclear and cytoplasmic details, clarity and uniform staining.

One study which used cedarwood oil as clearing agent has proven to have better results than the xylene. Their Statistical analysis as well showed significant correlation between nuclear staining (p value = 0.001); cytoplasmic staining (p value = 0.08) and background staining (p value = 0.045) indicating a positive results on using cedarwood oil as clearing agent [9].

In this review even though all three studies have shown positive results with usage of cedarwood oil, nobody has strongly recommended its usage in alternative to xylene. This could be because, As a natural product obtained from several

Table 1. Summary of the study characteristics of the articles included in this review

S.no	Author	Year	Sample size	Usage of cedarwood oil	Parameters analyzed	Scoring criteria	Statistical analysis	Results	Conclusion	Limitations
1.	K, Sithara & B, Ganesh & Desai, Dinkar	2021	50	Deparaffinizing agent	I. Cellular architecture II. Staining quality	1. Cellular architecture: 1) SCORE 0: Indistinct nucleus - cytoplasm 2) SCORE 1: Distinct Nucleus – cytoplasm 2. Quality of staining: 1) SCORE - 0 = Poor 2) SCORE - 1 = Satisfactory 3 3) SCORE - 2 = Good	Nil	Cellular architecture was found to be 74% accurate with Cedar wood oil. 42% of the samples showed good quality of staining.	This study concluded with xylene as the best deparaffinizing agent. Though Xylene is toxic, it is still the best deparaffinizing agent than the natural agents.	The staining quality assessment which was done by a single observer. No standardized protocol for natural substitutes.
2.	Thamilselvan,S et al.	2021	25	Clearing agent	I. Nuclear staining II. cytoplasmic staining III. Background staining IV. artifacts	1. Nuclear and cytoplasmic staining: (1) Poor Staining with poor morphology, (2) fair staining, (3) good staining and (4) excellent staining. 2. Background staining: (0) Nil, (1) mild, (2) moderate and (3) sever background staining. 3. Artifacts: Presence/absence of artifacts.	The scores of the samples processed by the clearing method between xylene and cedarwood oil were compared with Mann–Whitney U-test. The subgroup analysis of individual tissues was done using Kruskal–Wallis ANOVA.	The results of our study on comparison showed better outcome in tissues processed with cedarwood oil than xylene. Statistical Significant correlation was observed in nuclear staining (p value = 0.001) ;	The cedarwood oil can be considered as a safer natural alternative to xylene in laboratories.	Much longer processing time and also not cost-effective, more viscous.

S.no	Author	Year	Sample size	Usage of cedarwood oil	Parameters analyzed	Scoring criteria	Statistical analysis	Results	Conclusion	Limitations
								cytoplasmic staining (p value = 0.08) and background staining (p value = 0.045) indicating a positive results on using cedarwood oil as clearing agent.		
3.	Indu S, et al.	2014	30	Deparaffinizing agent	I. Nuclear and cytoplasmic details II. Clarity III. Uniformity of staining	I. Nuclear staining (adequate = score 1, inadequate = score 0) II. Cytoplasmic staining (adequate = score 1, inadequate = score 0) III. Uniformity of staining (present = score 1, absent = score 0).	Percentage adequacy of the staining method between cedarwood and xylene was compared using Chi-square test.	Significant correlation was observed between cedarwood oil and xylene in terms of the three staining quality parameters assessed	The present study shows that xylene free H and E staining procedure carried out using an essential oil (8% cedarwood oil) produced quality staining with sufficient clarity and uniformity of staining.	Over a considerable period of time its suitability for stability in the staining of tissue sections.

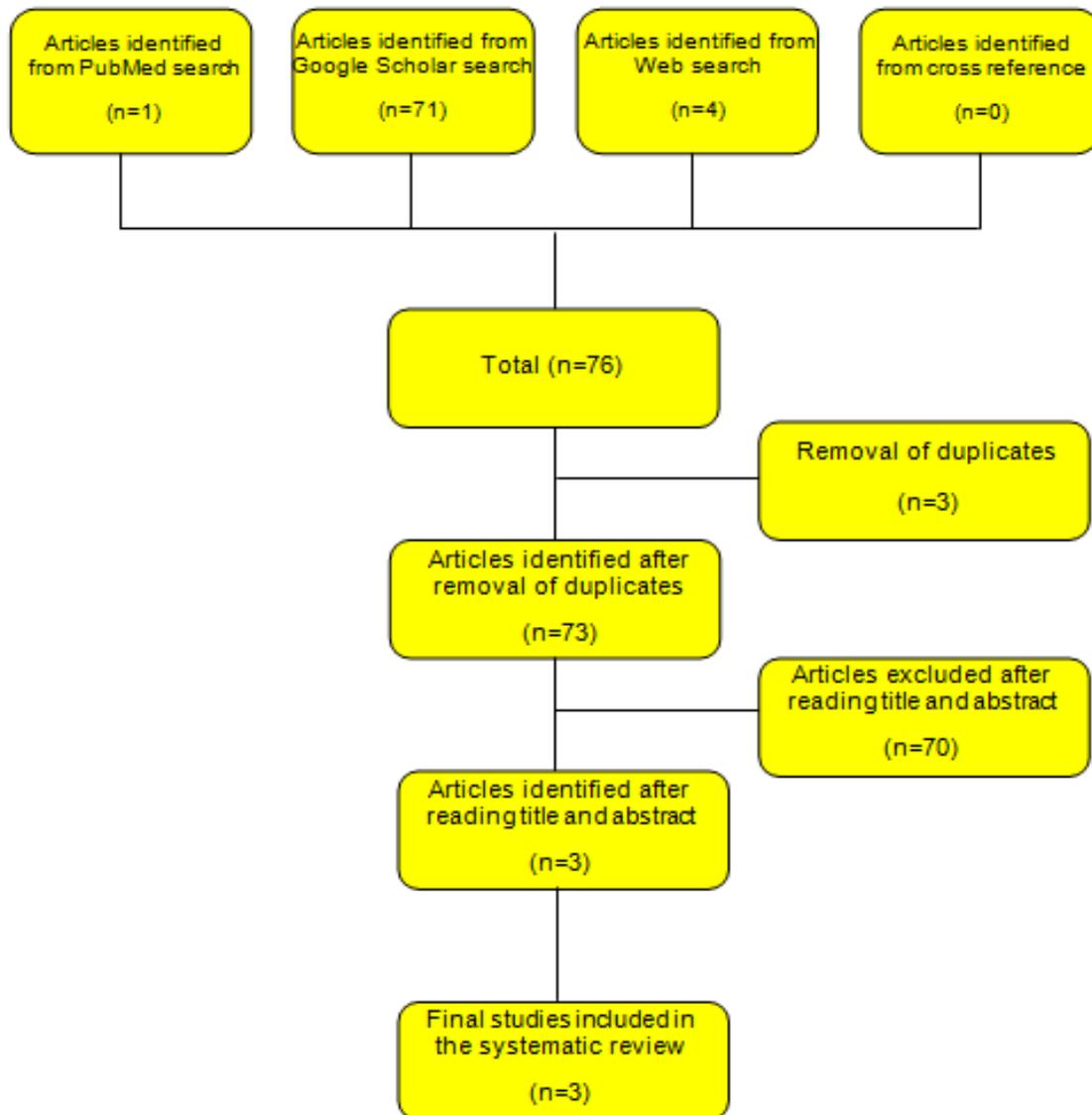


Fig. 1. A PRISMA flowchart illustrating the article selection process for this review

sources, characteristics and quality of the cedarwood oil differs. In regards with the histopathological diagnosis, two pivotal roles is that the tissues should not be distorted by any means during the processing and steps further which is completely satisfied by the cedarwood oil. It is gentler on the tissues. The clearing time will vary greatly, with more viscous oils taking longer than less viscous oils. Owing to the time consideration, it is always important to process the tissues as quick as possible so as to obtain the final diagnosis. Also the delay in the processing time would further delay the interpretation. The patients who have given the biopsy would have to remain anxious, even longer. For xylene the situation is vice versa.

Xylene is able to process tissues sooner with mild distortion of the tissues. Merits and demerits are equally found in both the reagents but compromising the time and cost in terms of cedarwood oil for health benefits hold good for the pathologists but bad for the patients. In case of xylene, compromising the health benefits of workers which results in quicker diagnosis hold good for the patients but not for the pathologists. Finally storage and disposal of these two reagents still remains questionable.

5. CONCLUSION

The cedarwood oil has produced considerable and acceptable results in comparison with xylene

as clearing and deparaffinizing agent. Yet, more innovations are required on larger scale with cedarwood oil for efficient usage with minimal duration for processing and dewaxing in histopathological laboratories.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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