Review Article

Oil as an Alternative Clearing Agent to Xylene in Histological Staining Process: A Systematic Review

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ABSTRACT

Introduction: Clearing in tissue processing removes alcohol and allows the tissue to be infiltrated with paraffin wax. It renders the tissue transparent, making it easier to be examined under the microscope. The most commonly used clearing agent in the laboratory is xylene. However, despite being proven as an effective clearing agent, xylene is still extremely hazardous and could endanger one's health.

Aim of the Work: The aim of the study is to do a systematic review on the potential of plant-based oils to be an alternative to xylene in the histopathological clearing process.

Materials and Methods: Articles for this review were searched from PubMed, ScienceDirect, ResearchGate, and Google Scholar using the combination of the terms oil, plant-based, vegetable, clearing agent, alternative, xylene, cedarwood oil, palm oil, eucalyptus oil, carrot oil, olive oil, pine oil, rose oil, clove oil, groundnut oil, and coconut oil. Using the PRISMA flowchart and the established inclusion criteria, a total of twenty-two (22) studies were included. Data were then extracted, verified, and interpreted.

Results: Majority of the studies showed that oils such as coconut, olive, carrot, rose, pine, groundnut, and palm oils are comparable to xylene. Tissues treated with these oils, if not better, showed a similar capability to xylene in producing a good quality of staining, gross tissue changes, and cellular architecture after the clearing process.

Conclusion: Plant-based oils have the potential to be bio-friendly alternative to xylene in the histopathological clearing process. However, further investigation involving a wide array of specimens, storage, incubation, and other techniques is recommended to address the minor inconsistencies observed in the results.

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Key Words: Alternative, clearing agent, histopathology, plant-based oil, xylene.

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INTRODUCTION

In the histopathology laboratory, human tissues are sliced thinly to be processed in several procedures to focus on the abnormalities present on the tissues being observed. It becomes almost always colorless after being sliced by a microtome which poses a challenge for pathologists and histologists to identify the structure and the abnormalities embedded within the tissue. As such, staining procedures are performed to differentiate tissues from one another. However, these procedures do not only pause at staining the tissues due to the presence of alcohol. In order to remove the alcohol portion of the tissue, clearing agents must be used[1].

Clearing agents remove alcohol and other dehydrants from the tissue to make it receptive to the infiltration material, paraffin[2]. It lessens light scattering and makes the tissue transparent by reducing the refractive index mismatch between biomolecules, commonly lipids, and their surrounding media[3]. This makes the slide containing the tissue specimen to be easily read.

The most commonly used clearing agent in histopathology is Xylene. It is a naturally occurring, colorless, and sweet-smelling substance usually found in gas or petroleum, which became the most feasible substitution to chloroform, benzene, and toluene – all posing carcinogenicity to the laboratory personnel[4]. However, despite the advantages it brought in the processing of tissues, numerous studies revealed that this chemical is highly toxic, flammable, and carcinogenic, posing severe long-term health risks to technicians and pathologists when exposed over a prolonged period of time[5]. It was during dewaxing of sections where exposure to xylene is the greatest. This can occur in several ways – primarily through inhalation, ingestion, and eye or skin contact[6]. Moreover, it was also claimed that several parts of the body gets affected by xylene, including the central nervous system, cardiovascular system, skin, kidney, liver,

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and many other organs, which also leads to secondary infections[7]. Additionally, since human tissues are subjected to xylene, removal of alcohol means that the tissue shrinks such as fats are also removed. Overexposure to xylene than the intended time will cause tissue degradation, making the tissue unfit for observation in pathology[8].

Due to the imposing risk of xylene, there are other alternatives or substitutes proposed as a clearing agent. The features that must be followed by these clearing agents to become a good alternative to xylene are the following: removes alcohol, clears quickly without the tissue becoming brittle, preserves the tissue after dyeing, evaporates after a lengthy time, and, most importantly, prevents personnel from acquiring illnesses[9]. With these features, oil could be fitted to be an alternative clearing agent.

In a laboratory setting, healthcare professionals and personnel are exposed to xylene, a toxic chemical agent. With this, the researchers aim to conduct a systematic review that would explore the potential of oils, specifically, plant-based oils to be alternative clearing agents. This systematic review intends to discuss the potential usage of various oils as clearing agents during tissue processing, as well as their comparability with xylene. Moreover, the efficacy of plant-based oils as alternative clearing agents and a substitute for xylene will be assessed based on peer-reviewed publications in scientific sites with strong methodology, comparative analysis of plant-based oil to xylene, and those that are relevant within ten (10) years.

MATERIALS AND METHODS

PRISMA Protocol

This systematic review adhered to the PRISMA protocol by utilizing a 27-item checklist and PRISMA flow chart. The checklist covers all elements from the title up to the results and discussion of the study. With this, the authors were guided in critically assessing the completeness of information, reducing bias, and verifying the methods and findings of the related studies. Moreover, integrating the PRISMA flowchart provided a graphical representation of the study selection process, including the number of identified records, records screened, retrieved, excluded, and studies included. Thus, all these methods contributed to the quality and transparency of this systematic review.

Search Strategy

Following PRISMA protocol, the data for this study were gathered via esteemed electronic archives and online databases like PubMed, ScienceDirect, ResearchGate, and Google Scholar. The inquiry took place in the first quarter of 2024 using the combination of the terms:

1. oil,
2. plant-based oil,
3. alternative,
4. clearing agent,
5. xylene,
6. cedarwood oil,
7. palm oil,
8. eucalyptus oil,
9. carrot oil,
10. olive oil,
11. pine oil,
12. rose oil,
13. clove oil,
14. groundnut oil,
15. coconut oil.

Eligibility Criteria

Inclusion and exclusion criteria were established to assess the eligibility of the studies in this systematic review. Specifically, the studies that satisfy the following criteria were included: (1) Peer-reviewed, (2) Follows the PICO framework, (3) Published from 2013 to 2023, (4) Written using the English language, (5) Has an experimental study design, and (6) Presents methods and outcomes that focus on the effectiveness of plant-based oils as alternative clearing agents to xylene. On the other hand, studies were excluded if any of the following criteria is met: (1) Published before 2013, (2) Written in languages other than English, (3) Written as an editorial, review paper, systematic review or meta-analysis, (4) Contains incomplete text or data, and (5) Presents methods and outcomes that focus on the efficacy of non-oil products and oils that are not derived from plants as xylene substitute.

Selection Strategy

The researchers conducted a thorough and individual examination of all peer-reviewed publications falling within the defined scope and relevance. Following the removal of duplicates, the sourced articles were methodically reviewed, considering key components such as titles, abstracts, methodologies, and results to assess if they can be selected to be part of the study. Subsequently, each publication was evaluated based on the established eligibility criteria to determine its inclusion. The final analysis was submitted to the research adviser for validation, verification, and feedback on the information and data presented.

Data Extraction

The researchers utilized Microsoft Excel, a tool used to collect, manage, and document materials, to organize the sourced articles and to extract data from all the included studies. The information collected from the included studies are the following: title of the study, authors, publication year, study design, specimen used, alternative oil used, key findings (staining quality, gross changes, and cellular architecture), and recommendations. The data obtained were then analyzed to assess the potential of oils to be an alternative clearing agent to xylene.

Quality Assessment

The literary search on the publication included was carried out by five (5) independent reviewers (N.S., K.S., J.T., A.T., and T.T.). Based on the eligibility criteria, each of these reviewers deliberately selected, and extracted papers that met the study topic's relevance. These five reviewers utilized the JBI Critical Appraisal Tool for Quasi-Experimental Studies to critically appraise the included studies. It consists of nine (9) direct questions that can be answered by four options: yes, no, unclear, or not applicable. After evaluating the checklist, an overall appraisal of the article is available with the following options: include, exclude, and seek further information. In cases where the reviewers decided to exclude the article, a comment section to reason out the exclusion of the said study was also provided. Conflicts on the checklist were then verified and resolved by two (2) additional independent reviewers (A.S., and S.S.) based on the full-text articles.

Data Analysis

The data extracted from the included studies were analyzed using a qualitative approach. Comparison and contrast between the findings of each publication were conducted to have a collective understanding of the topic, and to assess their areas of agreement or disagreement with regards to the use of oil as a clearing agent. The data analyzed were then summarized and presented as the result of this review, serving as basis for recommendations.

RESULTS

Search Results

The PRISMA flow diagram in Figure 1 provides an overview of the study selection process. 606 records were identified through PubMed, ResearchGate, ScienceDirect, and Google Scholar. After removing duplicates, 472 journals were screened based on their titles and abstracts related to the use of plant-based oils. Then, 337 studies were excluded as they did not meet the inclusion criteria. Subsequently, 135 full-text articles were assessed for eligibility, resulting in the exclusion of 113 journals that did not focus on the use of oils, particularly plant-based oils, as an alternative clearing agent to xylene. Finally, 22 studies which covered various experimental approaches were included in the systematic review, specifically 5 from PubMed, 1 from ScienceDirect, 8 from ResearchGate, and 9 from Google Scholar (Figure 1).

Figure 2 presents an overview of the twenty-two research studies included in this review, with a focus on India, Indonesia, Pakistan, Nigeria, and Brazil. Among these, sixteen studies originating from Indonesia are delineated in red which were authored by Digala *et al*., Swamy *et al*., Ravindran *et al*., Madhura *et al*., Thamilselvan *et al*., Indu *et al*., Sinha *et al*., Sermadi *et al*. (2014), Sermadi *et al*. (2019), Chandraker *et al*., Ashitha, A., Saravanakumar *et al*., Bordoloi *et al*., Sravya *et al*., Ghosh *et al*., and Tanwar *et al*. Consequently, Nigeria is represented by three studies highlighted in blue, authored by Adeniyi *et al*., Tsamiya *et al*., and Akpulu, S. P. Lastly, one study each from Pakistan in orange, Indonesia in brown, and Brazil in purple are highlighted which are authored by Manzoor *et al*., Rahmawati *et al*., and de Abreu *et al*., respectively (Figure 2).

Staining Quality and Resolution of Tissues After Clearing

The widespread use of coconut oil as the primary focus in investigations as an alternative to xylene in histological staining processes prompts compelling inquiries into its effectiveness. In India, Digala *et al*. (2019) revealed that in terms of overall staining quality, coconut oil is equivalent to xylene as they both garnered a score of two (2), which interprets to a good quality of staining[16]. This is similar to the scientific experiment of Saravanakumar *et al*. (2019) which presented a 100% staining quality of coconut oil parallel to xylene[22]. Bordoloi *et al*. (2022) also reported the same results after investigating the clearing capabilities of a specific brand of coconut oil profusely patronized in India. Notably, Sermadi *et al*. (2014) found that sections treated with coconut oil showed no appreciable change in the quality of the staining or cellular features[23]. Furthermore, the research by Ashitha *et al*. (2018) further supports the benefits of coconut oil by demonstrating superior characteristics when compared to palm oil, especially with regard to stiffness, translucency, and changes during impregnation. The sections treated with coconut oil showed better overall staining clarity, cytoplasmic staining, and nuclear staining[12]. The findings of Sinha *et al*. (2022) support the mounting body of evidence by showing that specimens treated with coconut oil and olive oil showed comparable characteristics to xylene at the macro and microscopic levels, including better nuclear staining, cytoplasmic staining, and overall staining quality[25]. The results obtained from many investigations collectively indicate that coconut oil has the potential to serve as an affordable and non-toxic alternative to xylene in histopathology laboratories.

In terms of olive oil, the findings of the study conducted by Tsamiya *et al*. (2021) shows that it was able to clear sections of liver, but not kidney[28]. Sinha *et al*., (2022) further evaluated this oil in another study, which was scored as zero (0), indicating poor quality of staining[25]. However, Swamy *et al*. (2015) scored olive oil's quality[7,24] of staining as one (1), indicating satisfactory, whereas Sermadi (2019) inferred that it does not have any adverse impact on stain quality. It is also important to note that in another study, extra virgin olive oil had a very good quality of staining[4]. Five (5) of the studies also tested out palm oil, which yielded the same results of the said oil having a similar quality of staining with xylene. Moreover, another type of oil that was used in four (4) of the included studies is groundnut oil, which is said to have a similar quality of staining with xylene[16]. An experiment by Tsamiya *et al*. (2021) states that groundnut oil has a superior quality of staining, similar to the findings of Saravanakumar (2019)[28]. Similarly, Adeniyi (2016), demonstrated that the same oil can be an alternative to xylene, as it was able to clear wood sections successfully[10]. Furthermore, cedarwood oil was also tested in three (3) of the articles, which scored over 90%, showing adequacy in the study of Indu *et al*. (2014)[17]. Similarly, Manzoor (2022) evaluated the same oil, which resulted to similar results.

Furthermore, Akpulu (2021) showed that the staining quality of Eucalyptus oil demonstrated good contrast in comparison with Xylene[11]. While the refined sunflower oil is not considered better than xylene in most aspects, it showed adequacy in clearing and deparaffinizing tissues and garnered a score of three (3) in the evaluation of staining quality, equating to good quality[4]. Moreover, a study conducted by Sravya *et al*. (2018) established sesame oil as a safer alternative to Xylene when processed with 95% diluted lemon water and 1.7% dish washing solution, as it demonstrated superior staining quality, scoring over 88.7% and 78% respectively[26]. As for the corn oil, brighter staining was demonstrated and it garnered two scores of two (2) in the quality of staining for kidney and liver, which equates to very good. Lastly, Swamy *et al*. (2015) compared four different oils: carrot oil, olive oil, pine oil, and rose oil. Among the aforementioned oils, pine oil scored the highest for the quality of staining and clearing properties. Whereas, the three other oils demonstrated only a satisfactory quality of staining[7]. Nonetheless, all four oils showed no significant difference in the quality of staining and thus, can be considered as an alternative for Xylene (Table 1).

Gross Tissue Changes After Clearing

Following tissue clearing, most of the included studies evaluated the gross tissue changes that occurred among the samples. This involves the changes in terms of translucency, rigidity, impregnation, ease of section cutting, and shrinkage (Table 2).

Translucency

Findings of this systematic review reveal that in terms of translucency, coconut oil recorded an inconsistent result across different studies. Digala *et al*. (2019) showed that coconut oil-processed tissue samples have the same translucency when compared to xylene, as all the tissues cleared with these two agents obtained a score of 1[16]. This is in line with the study of Tanwar *et al*. (2022) wherein 94.3% of the coconut oil-processed tissues were found to be translucent, similar to those processed using xylene which obtained the same percentage of translucent tissues[27]. Chandraker *et al*. (2019), however, had a different outcome as they concluded that the tissues cleared with coconut oil were less translucent[14]. This is similar to the findings of Adeniyi *et al*. (2016) wherein coconut oil was found to be the least transparent compared to xylene, groundnut oil and palm kernel oil; and findings of de Abreu *et al*. (2023) wherein the higher melting point of coconut oil was found to be the probable cause[10,15]. While these studies have opposing results, Rahmawati *et al*. (2020) had both outcomes in their study as the tissues obtained from kidney showed less translucency while those obtained from the liver showed the same translucency as xylene[20]. Contrary to these, the findings of Sermadi *et al*. (2014) showed that 100% of the tissues processed using coconut oil have a better translucency than those processed using xylene. Sinha *et al*. (2022) and Ashitha (2018) had the same conclusion, with the latter showing that majority (63.33%) of the coconut oil-processed samples were found to be more translucent than those of xylene[12,25].

Aside from coconut oil, other plant-based oils have also been assessed by other studies. Obtaining a score of 1, tissues treated with olive oil were found to have the same translucency compared to those treated with xylene[7,24]. Likewise, carrot and rose oil also obtained the same translucency to xylene after clearing. Compared to these three oils, pine oil was found to be superior as it was given a score of 2 which means that it has a better capacity than xylene in making the tissue samples translucent[7]. Moreover, tissues processed using groundnut oil obtained a score of 2 as well, making it superior to xylene, and a better alternative than coconut oil[16]. For palm oil, Madhura *et al*. (2016) showed that 100% of the tissues cleared with it have the same translucency as those of xylene[18]. While this is similar to the study of Ravindran *et al*. (2018) wherein 95% of the palm oil-processed samples showed no significant difference with xylene, this contradicts the findings of Ashitha (2018) wherein more than half (53.33%) of the samples showed better translucency[12,21].

Rigidity

Tissue samples processed using olive oil were less rigid than those processed using xylene. This is consistent with the study of Sinha *et al*. (2022), as well as Sermadi *et al*. (2019), which showed that majority (61.70%) of the specimen processed with olive oil obtained a score of 0, thus showing less rigidity than that of xylene[24,25]. Along with carrot, pine, and rose oil, this was also the findings of Swamy *et al*. (2015), wherein it received a grade of 0[7]. Similarly, although a different kind of oil, groundnut oil was reported to be inferior to xylene when it comes to rigidity[16]. Moreover, there are also oils that were reported of having the same rigidity as xylene. Rahmawati *et al*. (2020) revealed that palm and corn oil are equivalent to xylene in terms of rigidity as all kidney and liver tissue specimen obtained a score of 1[20]. Ashitha (2018) also arrived at the same conclusion as most (40%) of the palm oil-treated specimen recorded the same rigidity with those processed with xylene[12].

Same with translucency, inconsistencies were also recorded among the studies involving coconut oil. According to the study of Sermadi *et al*. (2014), majority (73%) of the coconut oil-processed tissue samples were found to be less rigid than those processed with xylene[23].

This agrees with the findings of Tanwar *et al*. (2022), Chandraker *et al* (2019), Sinha *et al*. (2022), and Digala *et al*. (2019). However, these are in contrast with the result of the studies conducted by Rahmawati *et al*. (2020) wherein all the kidney and liver tissue specimen processed with coconut oil had the same rigidity as to that of xylene, and Ashitha (2018) wherein most (43.33%) of the tissues treated with coconut oil and xylene showed no difference with one another[12,20].

Impregnation

Similar to xylene, 100% of the samples processed using coconut oil exhibited no change in impregnation[23]. This is consistent with the study of Ashitha (2018), wherein 90% of coconut-oil treated specimen showed the same change as to that of xylene[12]. This is in contrast, however, with the findings of Chandraker *et al*. (2019) wherein the tissues that were processed with coconut oil were found to be softer than the samples processed with xylene after wax infiltration[14]. Moreover, most (83.33%) of the palm oil-treated specimen and 100% of the olive oil-treated specimen exhibited a similar change with xylene after impregnation[12,24].

Ease of Section Cutting

It was shown that there is no significant difference in ease of sectioning between coconut oil and xylene[13]. This was supported by Sermadi *et al*. (2014) stating that coconut oil and xylene had the equal ease of sectioning[23]. Similarly, Tanwar *et al*. (2022) showed that there is no difference between the nicks and ribbons of both xylene and coconut oil[27]. In contrast, Chandraker *et al*. (2019) stated that the tissues embedded with coconut oil were more difficult to cut due to the solidifying capability of the oil at room temperature[14]. Chandraker *et al*.'s results were opposed, however, by de Abreu *et al*. (2023) stating that coconut oil-treated specimens showed the greatest ease due to the miscibility of coconut oil with paraffin[15].

In comparison with other oils, coconut oil equates to both palm and corn oil when it comes to ease of sectioning. Ashitha (2018) revealed that 93.33% of specimens treated with palm and coconut oil demonstrated the same quality of sectioning as xylene[12]. This agrees with Rahmawati *et al*.'s findings wherein coconut, palm and corn oil obtained a similar score with xylene. In addition, when compared to groundnut oil, coconut oil together with xylene, is inferior in terms of section cutting[16].

Furthermore, Sermadi *et al*. (2019) showed that olive oil- and xylene-treated tissues garnered the same scores which mean they had the same ease of sectioning[24]. Similarly, Swamy *et al*. (2015) showed an equal effect of rose, carrot, olive, and pine oil as to that of xylene in section cutting while Madhura *et al*. (2016) revealed that all specimens had ease of sectioning with ribbon formation[7,18]. Akpulu (2021) compared eucalyptus oil and xylene, yet both variables managed to have almost the same percentage according to ease of sectioning[11].

Shrinkage

Coconut oil, along with groundnut oil, did not cause the tissues to shrink greatly as opposed to what xylene did[16]. Multiple studies support this as the findings of Sermadi *et al*. (2014), Chandraker *et al*. (2019), Bordoloi *et al*. (2022), and Tanwar *et al*. (2022) also revealed that the shrinking capacity of coconut oil is lesser than that of xylene[13,14,23,27]. Rahmawati *et al*. (2020), however, deduced that coconut oil, as well as palm and corn oil, has the same shrinking capacity as xylene after obtaining a score of 1[20].

In addition, Swamy *et al*. (2015) found out that while carrot, rose, and pine oil have a lesser shrinking capacity, olive oil scored the same as xylene in shrinking tissues[7]. This is in contrast with the study of Sermadi *et al*. (2019) wherein it showed that xylene greatly decreases the volume of the tissue specimen than olive oil; and Sinha *et al*. (2022) wherein it revealed that xylene shrinks the tissues greatly in comparison to olive and coconut oil[24,25].

Cellular Architecture After Clearing

Adeniyi at al. (2016) demonstrated that groundnut, coconut, and palm kernel oils were able to maintain the distinct histological architecture of tissues, the same way as xylene. Chandraker *et al*. (2019) corroborated these findings, showing that coconut oil displayed superior architecture, and nuclear and cytoplasmic staining than xylene[14]. Similarly, de Abreu et al. (2023), Sermadi *et al*. (2014), and Tanwar *et al*. (2022) confirmed the ability of coconut oil in maintaining tissue morphology, and nuclear and cytoplasmic details without interfering with different stains[15,23,27]. In contrast, Bordoloi *et al*. (2022) and Sinha *et al*. (2022) showed that coconut oil-cleared tissues displayed a superior quality of stain, overall morphology, and cellular detail than xylene-cleared tissues[13,25]. Ashitha (2018) had the same result, adding that coconut oil was also found to be superior to palm oil[12].

Alongside coconut oil, groundnut oil, palm and corn oils were revealed to have a comparable architecture, and nuclear and cytoplasmic staining as xylene[16,20,21,22]. This opposes the findings of Madhura *et al*. (2016) wherein bleached palm oil was found to be inferior to xylene[18]. Moreover, Swamy *et al*. (2015) revealed that tissues cleared with carrot, olive, pine and rose oils were similar to xylene in terms of architecture and morphology[7]. Sermadi *et al*. (2019) also reported the same for olive oil. However, while olive oil was found to be similar to xylene, it was revealed to be superior to sunflower oil[4] and inferior to groundnut oil along with clove oil[28].

For cedarwood oil, Indu *et al*. (2014) and Manzoor *et al*. (2022) showed that it has a comparable result to xylene in nuclear and cytoplasmic staining[12,19]. This is in contrast with the findings of Thamilselvan *et al*. (2021), showing that cedarwood oil is better than xylene. Furthermore, Sravya *et al*. (2018) examined sesame oil with different dilutions as an alternative. Tissues processed with sesame oil and stained using 1.7% DWS were found to be an effective alternative to xylene[26]. Lastly, Akpulu (2021) emphasized the effectiveness of Eucalyptus oil, showing that it was able to preserve tissue cellular architecture the same was as xylene (Table 3). The summary of the extracted data from all the studies included can be found in (Table 4).

Quality Assessment of the Included Studies

The result of the quality assessment of the twenty-two experimental studies is shown in (Table 5).

According to the JBI Critical Appraisal Checklist for Quasi-Experimental Studies, all twenty-two experimental studies met the majority of the tool's criteria, meeting six to seven out of nine possible questions. One out of nine items, pertaining to follow-up between compared groups and its loss, was not applicable to all studies due to their experimental nature, and no loss of follow-up was reported. Additionally, one out of nine items related to statistical analysis was not applicable to some studies, as they employed descriptive analysis. Hence, the overall appraisal of these experimental studies led to their inclusion in this review.

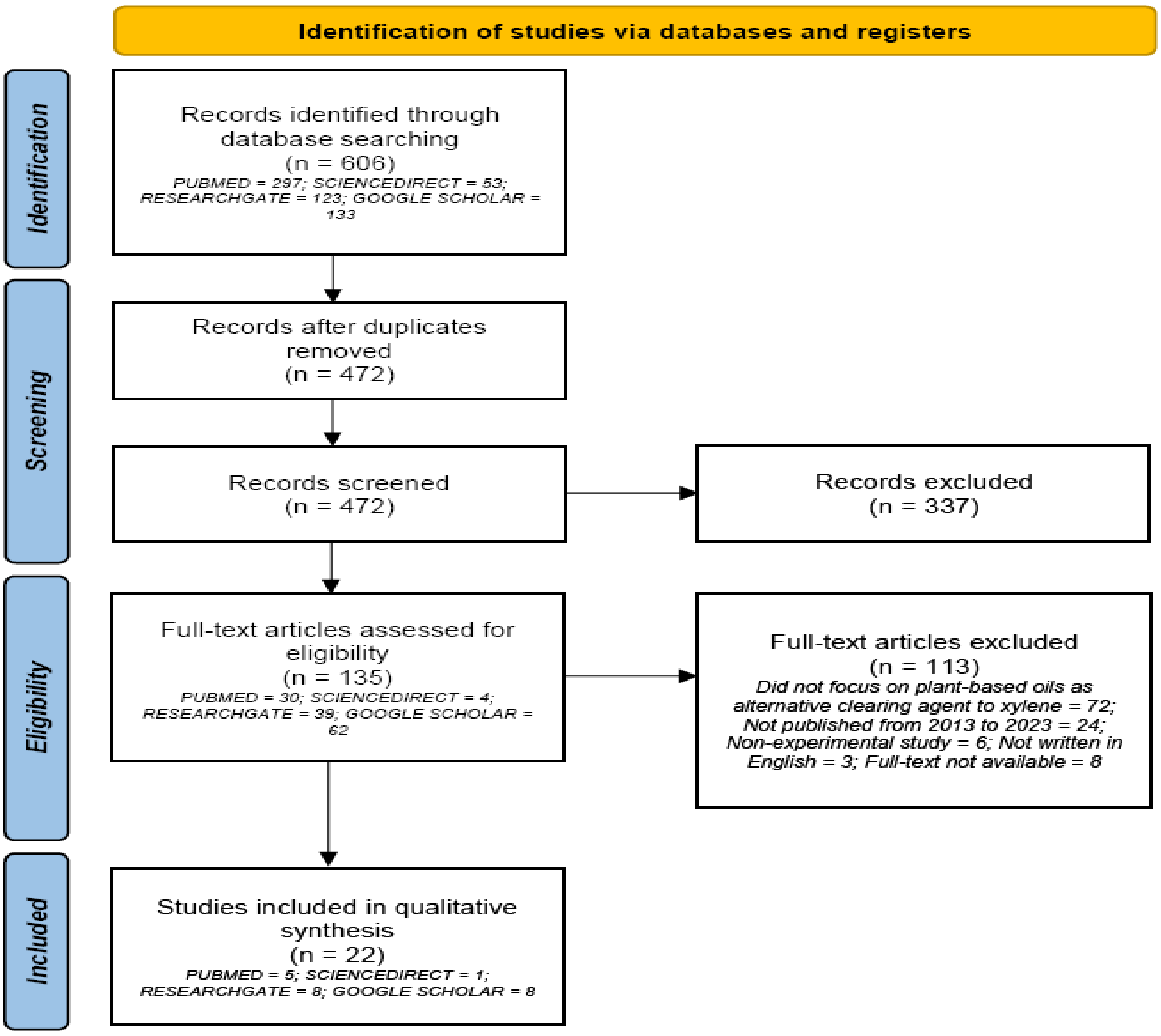

Fig. 1: Identification of studies via databases and registers

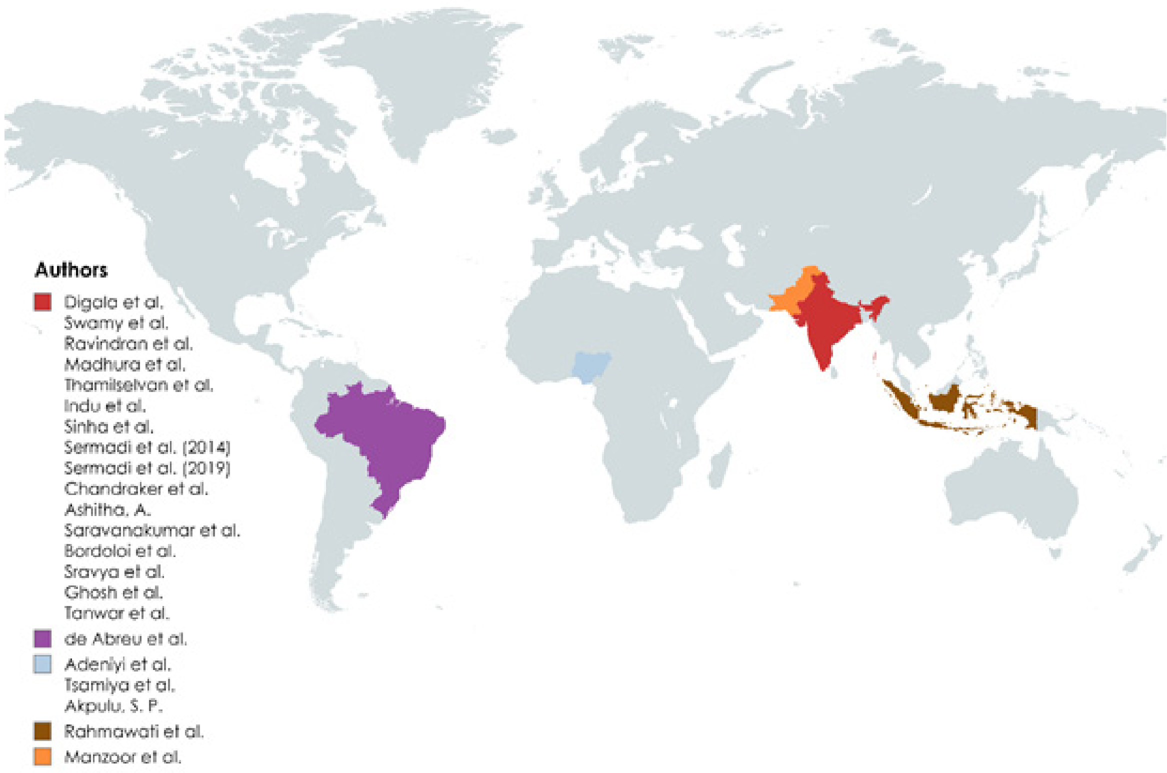

Fig. 2: Mapping of the Studies Included for the Review

Table 1: Staining Quality and Resolution of Tissues After Clearing

Authors (Publication Year)	Alternative Oil Used	Overall Quality of Staining
Adeniyi *et al.* (2016)	Groundnut Oil, Coconut Oil, Palm Kernel Oil	No significant differences in comparison to Xylene
Akpulu, S.P. (2021)	Eucalyptus (Citrodora) Oil	Better contrast than Xylene
Ashitha, A. S. (2018)	Coconut Oil & Palm Oil	Coconut oil has better staining quality than Palm oil; both has no significant differences in comparison to Xylene
Bordoloi *et al.*. (2022)	Coconut Oil	No significant differences in comparison to Xylene
Chandraker *et al.*. (2019)	Coconut Oil	No significant differences in comparison to Xylene
de Abreu *et al.*. (2023)	Extra Virgin Coconut Oil	No significant differences in comparison to Xylene
Digala *et al.*. (2019)	Coconut Oil & Groundnut Oil	Good quality
Ghosh *et al.*. (2016)	Extra Virgin Olive Oil & Refined Sunflower Oil	Extra virgin olive oil has better staining quality than RSO; both has no significant differences in comparison to Xylene
Indu *et al.*. (2014)	Cedarwood Oil	No significant differences in comparison to Xylene
Madhura *et al.*. (2016)	Bleached Palm Oil	Good and satisfactory
Manzoor *et al.*. (2022)	Cedarwood Oil	Satisfactory
Rahmawati *et al.*. (2020)	Palm Oil, Corn Oil & Coconut Oil	Good quality
Ravindran *et al.*. (2018)	Bleached Palm Oil	Good quality
Saravanakumar *et al.*. (2019)	Groundnut Oil & Coconut Oil	No significant differences in comparison to Xylene
Sermadi *et al.*. (2014)	Coconut Oil	No significant differences in comparison to Xylene
Sermadi *et al.*. (2019)	Olive Oil	No adverse impacts on staining quality in comparison to Xylene
Sinha *et al.*. (2022)	Coconut Oil and Olive Oil	No significant differences in comparison to Xylene
Sravya *et al.*. (2019)	Sesame Oil with 95% DLW (Group A) & Sesame Oil with 1.7% DWS (Group B)	Tissues in Group B stained better than in Group A; Sesame oil with 1.7% DWS less quality to same quality with Xylene
Swamy *et al.*. (2015)	Carrot Oil, Olive Oil, Pine Oil, & Rose Oil	Carrot oil, olive oil, and rose oil are satisfactory; Pine oil showed good staining quality
Tanwar *et al.*. (2022)	Coconut Oil	No significant differences in comparison to Xylene
Thamilselvan *et al.*. (2021)	Cedarwood Oil	Better staining quality than Xylene
Tsamiya *et al.*. (2021)	Clove Oil, Olive Oil & Groundnut Oil	Clove oil and Olive oil have satisfactory quality; Groundnut oil showed good staining quality

Table 2: Gross Tissue Changes in Comparison with Xylene

Authors (Publication Year)	Alternative Oil Used	Translucency	Rigidity	Impregnation	Ease of Section Cutting	Shrinkage
Adeniyi *et al.*. (2016)	Groundnut Oil, Coconut Oil, Palm Kernel Oil	Coconut oil: Inferior to xylene, groundnut, and palm kernel oil	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Akpulu, S.P. (2021)	Eucalyptus (Citrodora) Oil	Not evaluated	Not evaluated	Not evaluated	Similar to xylene	Not evaluated
Ashitha, A. S. (2018)	Coconut Oil & Palm Oil	Better than xylene	Similar to xylene	Similar to xylene	Similar to xylene	Not evaluated
Bordoloi *et al.*. (2022)	Coconut Oil	Not evaluated	Not evaluated	Not evaluated	Similar to xylene	Lesser than xylene
Chandraker *et al.*. (2019)	Coconut Oil	Inferior to xylene	Inferior to xylene	Inferior to xylene	Inferior to xylene	Lesser than xylene
de Abreu *et al.*. (2023)	Extra Virgin Coconut Oil	Inferior to xylene	Not evaluated	Not evaluated	Better than xylene	Not evaluated
Digala *et al.*. (2019)	Coconut Oil & Groundnut Oil	Coconut oil: Similar to xylene Groundnut oil: Better than xylene	Inferior to xylene	Not evaluated	Coconut oil: Inferior to xylene and groundnut oil	Lesser than xylene
Ghosh *et al.*. (2016)	Extra Virgin Olive Oil & Refined Sunflower Oil	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Indu *et al.*. (2014)	Cedarwood Oil	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Madhura *et al.*. (2016)	Bleached Palm Oil	Similar to xylene	Not evaluated	Not evaluated	Similar to xylene	Not evaluated
Manzoor *et al.* (2022)	Cedarwood Oil	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Rahmawati *et al.*. (2020)	Palm Oil, Corn Oil & Coconut Oil	Palm & corn oil: Inferior to xylene Coconut oil: Inferior (kidney), Similar (liver) to xylene	Similar to xylene	Not evaluated	Similar to xylene	Similar to xylene
Ravindran *et al.*. (2018)	Bleached Palm Oil	Similar to xylene	Not evaluated	Not evaluated	Inferior to xylene	Not evaluated
Saravanakumar *et al.*. (2019)	Groundnut Oil & Coconut Oil	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Sermadi *et al.*. (2014)	Coconut Oil	Better than xylene	Inferior to xylene	Similar to xylene	Similar to xylene	Lesser than xylene
Sermadi *et al.*. (2019)	Olive Oil	Similar to xylene	Inferior to xylene	Similar to xylene	Similar to xylene	Lesser than xylene
Sinha *et al.*. (2022)	Coconut Oil and Olive Oil	Better than xylene	Inferior to xylene	Not evaluated	Not evaluated	Lesser than xylene
Sravya *et al.*. (2018)	Sesame Oil with 95% DLW (Group A) & Sesame Oil with 1.7% DWS (Group B)	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Swamy *et al.*. (2015)	Carrot Oil, Olive Oil, Pine Oil, & Rose Oil	Carrot, olive & rose oil: Similar to xylene Pine oil: Better than xylene and the other three oils	Inferior to xylene	Not evaluated	Similar to xylene	Carrot, rose & pine oil: Lesser than xylene Olive oil: Similar to xylene
Tanwar *et al.*. (2022)	Coconut Oil	Similar to xylene	Inferior to xylene	Not evaluated	Similar to xylene	Lesser than xylene
Thamilselvan *et al.*. (2021)	Cedarwood Oil	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Tsamiya *et al.*. (2021)	Clove Oil, Olive Oil & Groundnut Oil	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated

Table 3: Cellular Architecture Changes in Comparison with Xylene

Authors (Publication year)	Alternative Oil Used	Maintained Cellular architecture	Nuclear staining	Cytoplasmic staining
Adeniyi *et al.*. (2016)	Groundnut Oil, Coconut Oil, Palm Kernel Oil	Similar to xylene	Not evaluated	Not evaluated
Akpulu, S. P. (2021)	Eucalyptus (Citrodora) Oil	Similar to xylene	Similar to xylene	Similar to xylene
Ashitha, A. S. (2018)	Coconut Oil & Palm Oil	Coconut oil: Superior to other oils	Coconut oil: Superior to other oils	Coconut oil: Superior to other oils
Bordoloi *et al.*. (2022)	Coconut Oil	Better than xylene	Better than Xylene	Better than Xylene
Chandraker *et al.*. (2019)	Coconut Oil	Similar to xylene	Similar to xylene	Similar to xylene
de Abreu *et al.*. (2023)	Extra Virgin Coconut Oil	Similar to xylene	Not evaluated	Not evaluated
Digala *et al.*. (2019)	Coconut Oil & Groundnut Oil	Similar to xylene	Similar to xylene	Similar to xylene
Ghosh *et al.*. (2016)	Extra Virgin Olive Oil & Refined Sunflower Oil	Extra virgin olive oil: Similar to xylene Refined sunflower oil: Inferior to extra virgin olive oil & xylene	Extra virgin olive oil: Similar to xylene Refined sunflower oil: Inferior to extra virgin olive oil & xylene	Extra virgin olive oil: Similar to xylene Refined sunflower oil: Inferior to extra virgin olive oil & xylene
Indu *et al.*. (2014)	Cedarwood Oil	Similar to xylene	Similar to xylene	Similar to xylene
Mahudra *et al.*. (2016)	Bleached Palm Oil	Inferior to xylene	Inferior to xylene	Inferior to xylene
Manzoor *et al.*. (2022)	Cedarwood Oil	Similar to xylene	Similar to xylene	Similar to xylene
Rahmawati *et al.*. (2020)	Palm Oil, Corn Oil & Coconut Oil	Similar to xylene	Similar to xylene	Similar to xylene
Ravindran *et al.*. (2018)	Bleached Palm Oil	Similar to xylene	Similar to xylene	Inferior to xylene
Saravanakumar *et al.*. (2019)	Groundnut Oil & Coconut Oil	Similar to xylene	Similar to xylene	Similar to xylene
Sermadi *et al.*. (2014)	Coconut Oil	Similar to xylene	Similar to xylene	Similar to xylene
Sermadi *et al.*. (2019)	Olive Oil	Similar to xylene	Similar to xylene	Similar to xylene
Sinha *et al.*. (2022)	Coconut Oil & Olive Oil	Better than xylene	Better than xylene	Better than xylene
Sravya *et al.*. (2018)	Sesame Oil with 95% DLW (Group A) & Sesame Oil with 1.7% DWS (Group B)	Group A: Inferior to Group B & xylene Group B: Similar to xylene	Group A: Inferior to Group B & xylene Group B: Similar to xylene	Group A: Inferior to Group B & xylene Group B: Similar to xylene
Swamy *et al.*. (2015)	Carrot Oil, Olive Oil, Pine Oil & Rose Oil	Similar to xylene	Similar to xylene	Similar to xylene
Tanwar *et al.*. (2022)	Coconut Oil	Similar to xylene	Similar to xylene	Similar to xylene
Thamilselvan *et al.*. (2021)	Cedarwood Oil	Better than xylene	Better than xylene	Better than xylene
Tsamiya *et al.*. (2021)	Clove Oil, Olive Oil, & Groundnut Oil	Groundnut oil: Superior to other oils	Groundnut oil: Superior to other oils	Groundnut oil: Superior to other oils

Table 4. Summary of the Extracted Data from the Included Studies

Authors (Publication Year)	Study Design	Specimen Used	Alternative Oil Used	Key Findings
Adeniyi *et al.*. (2016)	Experimental Design	Wood specimens from Nauclea diderrichii	Groundnut Oil, Coconut Oil, Palm Kernel Oil	Distinct and clear wood features; No occurrence of feature distortion and inadequate translucency
Akpulu, S.P. (2021)	Experimental Design	Liver, kidney, brain, and testicular tissues of Adult Male Wistar Rats	Eucalyptus (Citrodora) Oil	Less frequent changing of oil; No negative interference in immunoactivity of antibodies; No statistical difference with Xylene
Ashitha, A. S. (2018)	Experimental Design	Thirty (30) gingival tissue specimens obtained from patients undergoing removal of third molar	Coconut Oil & Palm Oil	More translucent; Similar rigidity; Better nuclear staining (coconut oil)
Bordoloi *et al.*. (2022)	Experimental Design	Forty-five (45) soft-tissue samples comprised of epithelial hyperplasia, hyperkeratosis, fibrous hyperplasia, dentigerous cyst, ameloblastoma, odontogenic fibroma and peripheral giant cell granuloma	Coconut Oil	No shrinkage; Less presence of tissue folds; Similar quality of staining and cellular architecture with Xylene
Chandraker *et al.*. (2019)	Experimental Design	Twenty-five (25) oral soft tissue samples	Coconut Oil	Less translucent; Soft in rigidity and impregnation; Difficulty in sectioning; Distinct cellular architecture; Similar quality of staining with Xylene
de Abreu *et al.*. (2023)	Experimental Design	Five (5) fragments of tissues of Rattus norvegicus (cerebellum, tongue, and ear)	Extra virgin coconut oil	No change in morphology; No interference with different stains
Digala *et al.*. (2019)	Experimental Design	Appendix, gall bladder and hemorrhoid human tissue samples collected randomly from Billroth hospitals	Coconut oil & Groundnut oil	More translucent (groundnut oil); Less rigid; Less shrinkage; Similar quality of staining with Xylene
Ghosh *et al.*. (2016)	Experimental Design	One hundred twenty (120) specimens of fresh goat tissue (buccal mucosa, lymph node, liver, and salivary gland)	Extra virgin olive oil & refined sunflower oil	Lesser quality of staining, cytoplasmic and nuclear details, and cellular outline; Similar integrity except refined sunflower oil
Indu *et al.*. (2014)	Experimental Design	Thirty (30) paraffin blocks of routine biopsy specimens	Cedarwood oil	Similar quality of staining with Xylene
Madhura *et al.*. (2016)	Experimental Design	Twelve (12) normal oral mucosal tissue obtained from patients undergoing minor oral surgery	Bleached Palm Oil	Similar translucency; Poorer clarity; Poorer nuclear and cytoplasmic staining
Manzoor *et al.*. (2022)	Experimental Design	Thirty (30) blocks of paraffin-embedded obtained from routine biopsy	Cedarwood Oil	Similar quality of staining with Xylene
Rahmawati *et al.*. (2020)	Experimental Design	Liver and kidney tissue samples collected from four (4) male white rats	Palm oil, Corn oil & Coconut oil	Similar quality of staining except coconut oil; Similar rigidity, shrinkage and section cutting; Less translucent except liver tissues cleared with coconut oil
Ravindran *et al.*. (2018)	Experimental Design	Twenty (20) formalin-fixed tissue sample from the Department of Oral Pathology	Bleached Palm Oil	Adequate clarity and intensity of nuclear, cytoplasmic and uniformity staining; Similar translucency
Saravanakumar *et al.*. (2019)	Experimental Design	Forty-five (45) soft-tissue samples obtained from oral cavity-buccal mucosa, salivary gland, and muscle	Groundnut oil & Coconut Oil	Similar quality of staining and cellular architecture with Xylene
Sermadi *et al.*. (2014)	Experimental Design	Sixty (60) soft tissue specimens from head and neck region	Coconut Oil	Less rigid; More translucent; No significant shrinkage; Similar staining quality and tissue architecture with Xylene; Clearing procedure used in the oil must be in an incubator to prevent the solidification at room temperature
Sermadi *et al.*. (2019)	Experimental Design	Sixty (60) soft tissue specimens obtained from oral surgical procedures	Olive oil	Similar impregnation, section cutting, translucency, cytoplasmic and nuclear details; Good staining quality; Less rigid
Sinha *et al.*. (2022)	Experimental Design	One hundred ninety-two (192) formalin-fixed soft tissue specimens from buccal mucosa, lymph node, liver, and salivary gland of goats	Coconut Oil and Olive Oil	Less rigid; Less shrinkage; More translucent; Better staining quality

Sravya *et al.*. (2018)	Experimental Design	Ninety (90) paraffin-embedded tissue blocks	Sesame Oil with 95% DLW (Group A) & Sesame Oil with 1.7% DWS (Group B)	Adequacy in the uniformity, clarity, and intensity, as well as nuclear and cytoplasmic staining in Group B; Less performance than Xylene
Swamy *et al.*. (2015)	Experimental Design	Forty (40) different tissue samples	Carrot oil, Olive oil, Pine oil, & Rose oil	Less rigid; Less shrinkage except olive oil; Maintains cellular structure; Pine Oil scoring higher in staining quality and translucency.
Tanwar *et al.*. (2022)	Experimental Design	Seventy (70) samples (gingival enlargements, odontogenic cysts, benign epithelial lesions, oral squamous cell carcinomas, salivary gland tumors, benign connective tissue tumors, mucocutaneous lesions)	Coconut oil	Less shrinkage and rigidity than xylene; No difference in translucency; Good quality of histological details
Thamilselvan *et al.*. (2021)	Experimental Design	Oral squamous cell carcinoma resection specimens	Cedarwood oil	Better results in nuclear and cytoplasmic staining; No damage to tissue specimens over time.
Tsamiya *et al.*. (2021)	Experimental Design	Liver, kidney, and heart sections of two (2) healthy Wistar rats	Clove oil, Olive oil & Groundnut oil	Less shrinkage; Maintains tissue architecture and staining quality of tissue sections; Groundnut oil is more preferred.

Table 5: JBI Critical Appraisal Checklist for Quasi-Experimental Studies

Authors	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Included
Adeniyi *et al.*. (2016)	Y	Y	Y	Y	Y	NA	Y	Y	NA	✓
Akpulu, S.P. (2021)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Ashitha, A. S. (2018)	Y	Y	Y	Y	Y	NA	Y	Y	NA	✓
Bordoloi *et al.*. (2022)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Chandraker *et al.*. (2019)	Y	Y	Y	Y	Y	NA	Y	Y	NA	✓
de Abreu *et al.*. (2023)	Y	Y	N	Y	Y	NA	Y	Y	NA	✓
Digala *et al.*. (2019)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Ghosh *et al.*. (2016)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Indu *et al.*. (2014)	Y	Y	Y	Y	N	NA	Y	Y	Y	✓
Madhura *et al.*. (2018)	Y	Y	N	Y	Y	NA	Y	Y	Y	✓
Manzoor *et al.*. (2022)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Rahmawati *et al.*. (2020)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Ravindran *et al.*. (2018)	Y	Y	N	Y	Y	NA	Y	Y	Y	✓
Saravanakumar *et al.*. (2019)	Y	Y	Y	Y	Y	NA	Y	Y	NA	✓
Sermadi *et al.*. (2014)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Sermadi *et al.*. (2019)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Sinha *et al.*. (2022)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Sravya *et al.*. (2018)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Swamy *et al.*. (2015)	Y	Y	Y	Y	Y	NA	Y	Y	NA	✓
Tanwar *et al.*. (2022)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Thamilselvan *et al.*. (2021)	Y	Y	Y	Y	Y	NA	Y	Y	Y	✓
Tsamiya *et al.*. (2021)	Y	Y	Y	Y	Y	NA	Y	Y	NA	✓

Y, yes; N, no; U, Unclear; NA, Not applicable. Q1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)? Q2. Were the participants included in any comparisons similar? Q3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest? Q4. Was there a control group? Q5. Were there multiple measurements of the outcome both pre and post the intervention/exposure? Q6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed? Q7. Were the outcomes of participants included in any comparisons measured in the same way? Q8. Were outcomes measured in a reliable way? Q9. Was appropriate statistical analysis used?

DISCUSSION

The findings of this systematic review presented that seven (7) of the oils evaluated display an almost similar clearing properties with xylene across different criteria. Although most of them scored lesser in rigidity, coconut, olive, carrot, rose, pine, groundnut, and palm oils were able to obtain the same score as xylene in terms of staining, translucency, impregnation and cellular architecture, and a better score in terms of shrinkage.

Notably, across the included studies, there was no standardized practice undertaken in terms of the specimen used, staining technique, and incubation period. This could have contributed to the observed inconsistencies in results among some of the included studies. Also, the criteria used by each of them varied. While the translucency, staining quality, and cellular architecture of the tissues after clearing were evaluated by the majority, a considerable number of studies did not include rigidity, impregnation, ease of section cutting, and shrinkage as criteria of a good clearing agent. Moreover, there were also studies where multiple oils were used as the subject of the experiment. Some of these studies were able to compare the oils to one another, revealing which one is superior or inferior, while some was not able to do the same and only compared the oils individually to xylene. With these, the information presented by the studies also varied.

Regardless, the collective evidence from these studies still underscores the potential of various plant-based oils as effective substitutes for xylene in the histopathological clearing process. Although it is challenging to point out which oil is better than the other due to the lack of comparisons made, the results were still able to give an idea on what oil, only in the context with its comparison to xylene, can be a bio-friendly alternative. This not only reduces occupational hazards but also ensures the integrity of tissues crucial for accurate histopathological diagnoses.

CONCLUSION

Despite being the commonly used clearing agent in laboratories, xylene poses risks and adverse health effects on humans due to its toxicity, carcinogenicity, and flammability. Having this, the results of this review emphasize the use of bio-friendly products such as oil as an efficient, effective, and safer substitute to xylene in the histopathological clearing process.

This review reveals that oils such as coconut, olive, carrot, rose, pine, groundnut, and palm oils are comparable to xylene in terms of the different parameters considered. Majority of them, if not better, were found to be similar to xylene in terms of translucency (olive, carrot, rose, pine, groundnut oil), rigidity (olive, carrot, rose, pine, groundnut, palm oil), impregnation (coconut, olive, palm oil), section cutting (coconut oil), shrinkage (coconut, olive, carrot, rose, pine, groundnut oil), and cellular architecture. Inconsistencies were mostly found among studies involving coconut oil especially in terms of translucency and rigidity. However, this may be attributed to the variations in the methodology that these studies employed. These differences, still, can serve as an opportunity to further investigate on what might have caused this, leading to the modification of the procedures used and the possible creation of standardized practices for the usage of oils as alternative clearing agents to xylene.

This review recommends the exploration of additional contributing factors, specifically the impacts of storage duration during staining, variations in mounting media, and the application of heat for shorter durations on tissues that have undergone clearing and dewaxing processes using plant-based oils, notably coconut oil, olive oil, groundnut oil, palm oil, etc. It is imperative as well to broaden the scope of investigation to encompass a diverse array of tissues, incorporating hard and decalcified tissues, tissues of disparate consistencies, and a spectrum of pathological diagnoses. This comprehensive approach is essential for deriving definitive conclusions regarding the comparative advantages of plant-based oil methodologies over conventional approaches.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الزيت كبديل للزيلين في عملية التلوين النسيجي: مراجعة منهجية

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مقدمه: تُزيل عملية التنقية في معالجة الأنسجة الكحول، وتسمح بتغلغل شمع البارافين في الأنسجة. كما أنها تجعل الأنسجة شفافة، مما يُسهّل فحصها تحت المجهر. يُعدّ الزيلين أكثر مواد التنقية استخدامًا في المختبر. ومع ذلك، ورغم ثبوت فعاليته، لا يزال الزيلين خطيرًا للغاية وقد يُشكّل خطرًا على الصحة.

الهدف من البحث : تهدف هذه الدراسة إلى إجراء مراجعة منهجية لإمكانية استخدام الزيوت النباتية كبديل للزيلين في عملية التنقية النسيجية المرضية.

مواد وطرق البحث : تم البحث عن المقالات المتعلقة بهذه المراجعة في PubMed وScienceDirect وResearchGate وGoogle Scholar باستخدام المصطلحات التالية: زيت، نباتي، نباتي، مادة تنقية، بديل، زيلين، زيت خشب الأرز، زيت النخيل، زيت الأوكالبتوس، زيت الجزر، زيت الزيتون، زيت الصنوبر، زيت الورد، زيت القرنفل، زيت الفول السوداني، وزيت جوز الهند. باستخدام مخطط PRISMA الانسيابي ومعايير الإدراج المُعتمدة، تم تضمين ما مجموعه اثنتين وعشرين (٢٢) دراسة. ثم استُخرجت البيانات، وتحققت منها، وفسرت.

النتائج : أظهرت غالبية الدراسات أن زيوتًا مثل جوز الهند، والزيتون، والجزر، والورد، والصنوبر، والفول السوداني، والنخيل تُضاهي الزيلين. أظهرت الأنسجة المُعالجة بهذه الزيوت، إن لم تكن أفضل، قدرةً مُماثلةً للزيلين في إنتاج صبغة عالية الجودة، وتغيراتٍ نسيجيةٍ واضحة، وبنيةٍ خلويةٍ بعد عملية التنقية.

خلاصة البحث: يُمكن للزيوت النباتية أن تُمثل بديلًا صديقًا للبيئة للزيلين في عملية التنقية النسيجية المرضية. ومع ذلك، يُوصى بإجراء مزيد من البحث يشمل مجموعةً واسعةً من العينات، والتخزين، والحضانة، وغيرها من التقنيات لمعالجة التناقضات الطفيفة المُلاحظة في النتائج.