

## OPTIMAL COMBINATION OF TOLUENE AND CLOVE OIL (*Syzigium aromaticum*) IN THE PROCESS OF CLEARING THE PERMANENT PREPARATION *PEDICULUS HUMANUS CAPITIS*

*Kombinasi Optimal Toluena dan Minyak Cengkeh (*Syzigium aromaticum*)  
dalam Proses Clearing Preparat Permanen *Pediculus Humanus Capitis**

**Bagus Muhammad Ihsan<sup>1\*</sup>, Vira Liesvia<sup>2</sup>, Hadits Lissentiya Armal<sup>2</sup>, Supriyanto  
Supriyanto<sup>1</sup>, Muhammad Ifham Hanif<sup>3</sup>**

<sup>1</sup>Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak,  
Indonesia

<sup>2</sup>Department of Medical Laboratory Technology, Poltekkes Kemenkes Banten,  
Indonesia,

<sup>3</sup>Badau Primary Health Care, Kapuas Hulu, Indonesia

\*Email: ihsanfillah24@gmail.com

### ABSTRACT

*Clearing is a vital process in microscopy, aimed at enhancing the transparency of tissue preparations for clearer observation of insect structures. Despite its effectiveness, Toluene poses safety concerns due to its explosive nature. Therefore, clove oil (*Syzygium aromaticum*) has been explored as a safer alternative. This study evaluates the quality of permanent preparations of *Pediculus humanus capitis* using both toluene and clove oil. Comparative tests were conducted, treating adult head lice specimens with each solvent for three repetitions. Permanent preparations were assessed for clarity, color quality, and morphological integrity. Results indicate a significant difference in quality between the two methods. Toluene yielded mainly poor-quality preparations, while clove oil resulted in a higher proportion of preparations of good quality. The Mann-Whitney test confirmed the significance of this difference ( $p = 0.002$ ). This study highlights the efficacy of clove oil as a safer alternative to toluene in the clearing process, contributing to entomological practices and chemical safety.*

**Keywords:** *clearing, clove oil (*Syzygium aromaticum*), *Pediculus humanus capitis*, permanent preparation quality*

### ABSTRAK

Clearing merupakan proses penting dalam mikroskopi yang bertujuan untuk meningkatkan transparansi preparasi jaringan sehingga struktur-serangga dapat diamati dengan lebih jelas. Toluena, meskipun efektif, menimbulkan kekhawatiran keselamatan karena sifatnya yang mudah meledak. Oleh karena itu, minyak cengkeh (*Syzygium aromaticum*) telah dieksplorasi sebagai alternatif yang lebih aman. Penelitian ini mengevaluasi kualitas preparasi permanen dari *Pediculus humanus capitis* menggunakan toluena dan minyak cengkeh. Tes komparatif dilakukan dengan memperlakukan spesimen kutu dewasa dengan masing-masing pelarut untuk tiga kali pengulangan. Preparasi permanen dinilai untuk kejernihan, kualitas warna, dan integritas morfologi. Hasil menunjukkan perbedaan yang signifikan dalam kualitas antara kedua metode tersebut. Toluena menghasilkan preparasi dengan kualitas yang sebagian besar buruk, sedangkan minyak cengkeh menghasilkan proporsi preparasi yang lebih tinggi dengan kualitas yang baik. Uji Mann-Whitney mengkonfirmasi signifikansi perbedaan ini ( $p = 0,002$ ). Penelitian ini menyoroti efikasi minyak cengkeh sebagai alternatif yang lebih aman bagi toluena dalam proses *clearing*, berkontribusi pada praktik entomologi dan keselamatan kimia.

**Kata kunci:** clearing, *Pediculus humanus capitis*, kualitas preparat permanen, minyak cengkeh (*Syzigium aromaticum*)

## INTRODUCTION

Making permanent preparations is one of the efforts of laboratory technicians to identify and see the morphology of parasites that disturb humans. One of the parasites that bother humans is *Pediculus humanus capitis*, head lice. Permanent preparations are declared inappropriate if there is an error at the preparation stage. One of the mistakes in the preparation stage can cause poor preparation quality, including color quality that is difficult to see, needs to be more transparent, and some of the morphology needs to be intact. Preparations do not last for a long time[1], [2]. Preparation of permanent preparations begins with immersion in 10% KOH (thinning of the exoskeleton), then dehydration (withdrawal of water molecules), clearing (clearing), and mounting (gluing tissue).

The clearing is a process that aims to clear the tissue preparation so that the structure is visible, clear, and transparent when observed under a microscope. Materials commonly used in clearing are xylol, benzol, toluene, acetone, and clove oil [3], [4]. The use of Xylol or Xylene in the manufacture of permanent preparations is a material that is commonly used in the clearing process; however, the use of xylol or xylene materials is included in hazardous and toxic chemicals and in the process of making permanent preparations of xylol solution can cause tissues to become dry and easily brittle[5], [6]. Based on previous research presented at the 2018 Unimus Student National Seminar on Differences in the Use of Xylol (Xylene) and Toluol (Toluene) in the Clearing Process on the Quality of Permanently Preserved Preparations *Cimex lectularis*, it was found that the preparation of permanently preserved preparations was clearer using toluol (toluene).

Agent compared to using xylol agent is toluene. However, the use of xylol and toluene are toxic materials found in anatomical pathology laboratories which can cause death either by ingestion, contact with the skin, or inhalation at specific concentrations and affect the nervous system[5], [8][7]. Therefore, they have to find other alternatives for using clearing materials in the manufacture of permanent preparations. Based on previous research by Fani Septiani in 2018 entitled Differences in Quality of Permanent *Pediculus humanus capitis* Preparations in the Clearing Process Using Xylol and Clove Oil, it was found that the use of clove oil was better than xylol.[1] Clove oil (*Syzigium aromaticum*) has advantages such as a faster clarification process in the clearing process, relatively low price, easy to obtain, and does not contain toxins[9]. Eugenol is the ingredient in clove oil (*Syzygium aromaticum*) that is linked to a quicker cleaning procedure. Clove oil's principal ingredient, eugenol, has qualities that speed up the process of clearing or clarifying when making permanent products[10].

Based on the problems above, the clearing process plays an essential role in the manufacture of permanent preparations for *Pediculus humanus capitis*. The use of toluene and clove oil (*Syzigium aromaticum*) solutions can produce good-quality endless preparations. The researcher intends to compare and see differences in the quality of permanent *Pediculus humanus capitis* preparations in the clearing process using toluene and clove oil (*Syzigium aromaticum*).

In addition to the aforementioned research findings, there are several other studies supporting the need for safer alternatives in the clearing process for permanent preparations. For instance, highlights the negative effects of toluene usage on the environment and human health, emphasizing the necessity to explore more environmentally friendly and safe alternatives [11]. Similarly, a study demonstrates that using clove oil as a clearing agent can yield more transparent preparations while maintaining morphological integrity [12]. This underscores the importance of further

research to explore the potential of clove oil as a safer and more effective alternative in the clearing process.

## **METHODS**

### **Research Design**

This study used a comparative test design, which means that the researcher treated both the subject and the object of the study. Specifically, the researcher compared the effects of toluene and clove oil (*Syzigium aromaticum*) on the quality of the permanent preparation of *Pediculus humanus capitis* during the clearing stage. Research ethics review no 06/KEPK-PK.PKP/V/D/2024

### **Research Location and Time**

The sampling location for clove oil (*Syzigium aromaticum*) was taken via e-commerce with several selected brands. The research was carried out in the parasitology laboratory, Department of Technology, Polytechnic Medical Laboratory, Ministry of Health, Pontianak. This research was conducted on November 2023-February 2024.

### **Research Population and Sample**

The population in this study was composed of several brands of clove oil (*Syzigium aromaticum*) The sample for this research used one of the brands of clove oil (*Syzigium aromaticum*). These oils' unique qualities and health advantages result from their composition, which includes terpenes, ketones, alcohols, aldehydes, and esters. They need very little to produce their scent because they are so strong and concentrated. Essential oils are found to be extensively used in aromatherapy, topical treatments following dilution, and as constituents in cosmetic and personal hygiene products. Essential oils can have a range of sticky, heavier textures or clear, liquid appearances depending on where they come from. They have a wide range of hues representing the pigmentation naturally found in the plant. Comprehending the multifaceted attributes of essential oils facilitates their secure and efficient employment in distinct contexts, endorsing general welfare and tranquility.

### **Tools and materials**

#### **Tool**

Microscope, screw bottle, nurse cap, petri dish, measuring cup, dropper pipette, Object glass, deck glass, beaker glass, stick, tissue

#### **Material**

The following things can be used to get rid of head lice: 10% KOH solution, Aquadest, graded alcohol (30%, 50%, and 96%), absolute alcohol, toluene, clove oil (*Syzigium aromaticum*), and Entellan.

### **How to collect data**

Data from direct observation and comparative research on permanent preparations for *Pediculus humanus capitis* in the clearing process using toluene and clove oil (*Syzigium aromaticum*). The research team involved in the research on the cleaning process of *Pediculus humanus capitis* using toluene and clove oil (*Syzigium aromaticum*) consists of the main researcher (2 people) and laboratory technicians (2 people): These researchers have a Master's degree (M.Sc.) in the field of entomology and parasitology with expertise in designing and applying scientific research methodologies, experience in the field of entomology, especially in the study of parasites such as *Pediculus humanus capitis* and skills in data analysis and scientific

writing, while for laboratory technicians have a Bachelor's education background (D4) in the field of Laboratory Technology and have practical skills in handling and processing biological samples, experience with laboratory equipment and chemicals such as toluene and clove oil as well as an understanding of cleaning techniques and permanent preparation for microscopic observations.

## **Research procedure**

### **Pre Analytics**

The process of taking *Pediculus humanus capitis* was carried out. In the process of taking *Pediculus humanus capitis* to make preparations, the collection is done by taking *Pediculus humanus capitis* from its place of origin (scalp or hair) directly using hands or without using tools, so as not to damage the body structure of *Pediculus humanus capitis* caused by fingers, combs tightly, or tweezers, Before taking *Pediculus humanus capitis*. Researchers should use a nurse's hat to protect themselves from pediculosis infection. 36 head lice (*Pediculosis humanus capitis*) are needed of the same size for 3 repetitions. Desired sample criteria: Large size: 3–4 mm, has intact morphology analytic.

### **Prepare research tools and materials.**

Use one slide for one sample, then Soak the *Pediculus humanus capitis* sample using 10% KOH for 24 hours, then rinse using distilled water. Soak *Pediculus humanus capitis* in a 30% alcohol solution for 15 minutes three times. Press with two glass objects to remove the fluid in the tick's stomach. Place the *Pediculus humanus capitis* sample in a 50% and 96% alcohol solution for 15 minutes each, and repeat three times. Place the *Pediculus humanus capitis* in an absolute alcohol solution for 5 minutes. The clearing process is done by putting the *Pediculus humanus capitis* sample in a different tub for 15 minutes with toluene and clove oil (*Syzigium aromaticum*). The mounting process is carried out, placed on a glass object treated with Canadian balsam, then covered with deck glass. Observed using a 4x objective lens magnification microscope to determine the color quality, clarity and morphological integrity of *Pediculus humanus capitis*[13].

### **Post Analytics**

An assessment was carried out on the quality of the comparisons that had been observed both macroscopically and microscopically. Assessment of Preparation Quality Results. Good quality preparations: clear preparations, without black spots, and the morphology of the *Pediculus humanus capitis* is intact. The quality of the preparation is quite good: the preparation is clear, there are black stains in  $< \frac{1}{4}$  of the preparation, and the morphology of the *Pediculus humanus capitis* is intact. Poor quality preparation: the preparation is not clear, there are black stains  $> \frac{1}{4}$  of the preparation, and the morphology of the *Pediculus humanus capitis* is damaged[13].

### **Data analysis**

Data were obtained by observing the morphology of adult *Pediculus humanus capitis* microscopically. The data is made in the form of a score against the assessment criteria, namely clarity, color quality and morphological integrity of *Pediculus humanus capitis* in permanent preparations. Each of these criteria is given a score of 1 if the clarity, color quality and integrity produced are poor. Score 2 if the clarity, color quality and integrity produced are quite good. Score 3 if the clarity, color quality and integrity produced are good. The score range includes poor criteria 1-3, quite good criteria 4-6, and good criteria 7-9[13]. The data obtained is presented in tabular form. Next, the data was put through the Mann-Whitney test to see if there was a significant difference between using toluene and clove oil (*Syzigium aromaticum*) to clear the area.

## RESULT

Based on the results of research that were conducted at the Parasitology Laboratory of the Pontianak Health Polytechnic on November 2023-February 2024, several brands of clove oil (*Syzigium aromaticum*) were obtained via E-commerce with several selected brands. The sample of this research uses one of the brands of clove oil (*Syzigium aromaticum*).

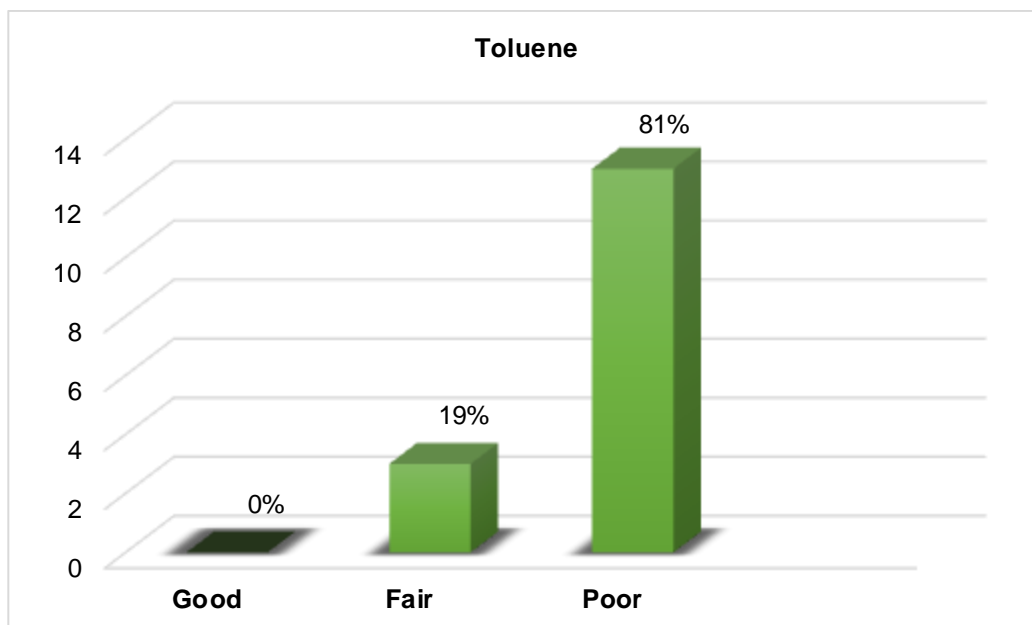


Figure 1. Graph of Quality Results of Permanent Preparations of *Pediculus humanus Capitis* Using Toluene

The clearing results in figure 1 based on the toluene solution on the chart obtained three permanent preparations of reasonably fair quality with a percentage of 19% and 13 endless practices of poor quality with a percentage of 81%.

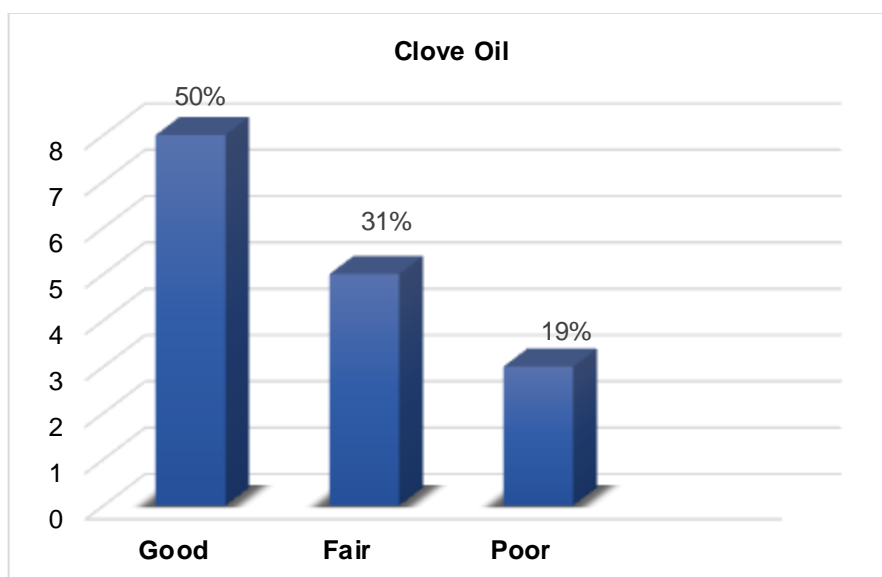


Figure 2. Graph of Quality Results of Permanent Preparations of *Pediculus humanus capitis* Using Clove Oil (*Syzigium aromaticum*)

The clearing results in based on the use of clove oil solution (*Syzigium aromaticum*) on figure 2 obtained eight good quality permanent preparations with a percentage of 50%, five endless preparations of reasonably fair quality with a percentage of 31%, three permanent practices of poor quality with a percentage of 19%.



**Figure 3. Observational Photos of Pediculus Humanus Capitis Preparations in Good Category with Toluene**

Figure 3. After observing under a microscope with an objective magnification of 4x, it was found that the results of the permanent preparations were in a fairly good category, showing clear preparations, there were black stains  $< \frac{1}{4}$  of the preparations, and the morphology of the *Pediculus humanus capitis* sample was intact, while the results of the *Pediculus humanus capitis* preparations were in a bad category with toluene can be seen in Figure 4.



**Figure 4. An Observational Photograph of The Setup Bad with Toluene: Pediculus Humanus Capitis Category**

Figure 4. After observing under a microscope with an objective magnification of 4x, it was found that the results of permanent preparations in the lousy category showed that the trials were not transparent, there were black stains  $> \frac{1}{4}$  of the practices, the morphology of the *Pediculus humanus capitis* sample was damaged, while the results of the *Pediculus humanus capitis* practices with no morphology complete and not transparent with toluene can be seen in Figure 5.



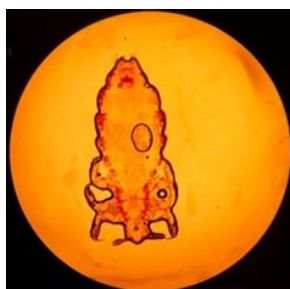
**Figure 5. Observational Photo of The Wrong Category of Pediculus Humanus Capitis Preparations with Incomplete Morphology and Not Transparent Using Toluene**

Figure 5. After observing under a microscope with an objective magnification of 4x, it was found that the results of the permanent preparations were incomplete morphologically, which was seen from the absence of the legs of *Pediculus humanus capitis* on the very front on the left and not transparent on the body of *Pediculus humanus capitis*. While the results of the preparation of *Pediculus humanus capitis* in the excellent category with clove oil can be seen in Figure 6.



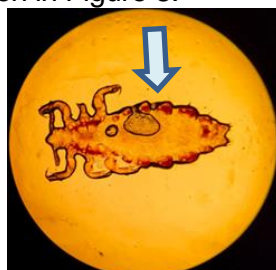
**Figure 6. Photo of a Good Category of *Pediculus humanus capitis* Preparations with Clove Oil (*Syzigium aromaticum*)**

Figure 6. After observing under a microscope with an objective magnification of 4x, it was found that the results of permanent preparations in the excellent category showed precise practices, without black stains, and the morphology of the *Pediculus humanus capitis* sample was intact so that every part of the body could be observed, while the results of *Pediculus humanus capitis* preparations with quite good category with clove oil (*Syzigium aromaticum*) can be seen in Figure 7.



**Figure 7. Observational Photos of *Pediculus humanus capitis* Preparations in Good Category with Clove Oil (*Syzigium aromaticum*)**

Figure 7. After observing under a microscope with an objective magnification of 4x, it was found that the results of the permanent preparations were in a pretty good category, showing precise trials, there were black stains  $< \frac{1}{4}$  of the practices, and the morphology of the *Pediculus humanus capitis* sample was intact, while the results of the *Pediculus humanus capitis* preparations were in a wrong category with clove oil (*Syzigium aromaticum*) can be seen in Figure 8.



**Figure 8. Photo of Poor Category of *Pediculus humanus capitis* Preparations with Clove Oil (*Syzigium aromaticum*)**

Figure 8. After observing under a microscope with an objective magnification of 4x, it was found that the results of permanent preparations in the lousy category showed that the trials were not transparent, there were black stains  $> \frac{1}{4}$  of the practices, the morphology of the *Pediculus humanus capitis* sample was damaged, and one of the legs was missing on the second part on the right.

Data from the image quality results of permanent preparations of *Pediculus humanus capitis* were then tested statistically. The statistical test used is the normality test and the Mann-Whitney test.

**Table 1. Normality Test**

	Reagent/Treatment Type	Significance Value (Shapiro-Wilk)
Preparation results	Toluene	0,484
	Clove Oil	0,768

Table 1 showed that the Normality test has a significance value for the use of toluene 0.484, while clove oil (*Syzigium aromaticum*) is 0.768. Because the significance value of both is greater than  $> 0.05$ , it can be concluded that the data from the permanent preparation of *Pediculus humanus capitis* are normally distributed.

**Table 2. Mann-Whitney test**

Treatment	Significant Value
Toluene	0,002
Clove Oil ( <i>Syzigium aromaticum</i> )	

Table 2 showed that the Normality test has a significant value for the use of toluene 0.484, while clove oil (*Syzigium aromaticum*) is 0.768. Because the significance value of both is more significant than  $> 0.05$ , it can be concluded that the data from the permanent preparation of *Pediculus humanus capitis* are normally distributed.

## PEMBAHASAN

The clearing is a process that aims to clear up permanent tissue preparations so that the observed morphological structures appear clear, clear, and transparent when observed with a microscope. Materials commonly used in the clearing process are benzol, toluene, xylol, and clove oil (*Syzigium aromaticum*).[3], [14]

The results showed differences between the treatments in the clearing process using toluene and clove oil (*Syzigium aromaticum*). Clearing clove oil (*Syzigium aromaticum*) is better than toluene in making permanent preparations. Clove oil (*Syzigium aromaticum*) contains 95% eugenol, one of the compounds in eugenol is a carbon group that functions in the dealcoholization process.[1], [15]

Dealcoholization is when the carbon groups in clove oil (*Syzigium aromaticum*) absorb the remaining alcohol from the dehydration process. Absorbing alcohol that is carried out ideally can make permanent tissue preparations produce good quality preparations. The eugenol content in clove oil (*Syzigium aromaticum*) of 95% may make the *Pediculus humanus capitis* sample look clearer and more transparent compared to a toluene solution during the clearing process, which contains less carbon, namely 90.5%.[16]–[18]

Based on Figure 3, it was found that the quality was not clear, there were black stains  $> \frac{1}{4}$  of the preparation, and the morphology of the *Pediculus humanus capitis* sample was damaged. This is because the clearing process is imperfect, which makes the body of the *Pediculus humanus capitis* sample still contain alcohol, so it cannot provide results that clearly show the structure of the morphology of the *Pediculus humanus capitis* sample.



In the clearing process using toluene, results were found from *Pediculus humanus capitis* samples whose morphology was incomplete. In this study, soaking *Pediculus humanus capitis* samples in a solution of toluene and clove oil (*Syzigium aromaticum*) was carried out based on a minimum clearing time of 15 minutes with three repetitions. Meanwhile, according to previous research regarding the effect of variations in toluene clearing time on the quality of *Cimex lectularis* preparations, it was found that the clearing time using the agent toluol (toluene) was 5 minutes without repetition.[16], [19], according to research on the differences in the quality of permanent preparations for *Pediculus humanus capitis*, the clearing process using xylol and clove oil is 10 minutes with three repetitions. According to research on variations in KOH concentration and clearing time on the quality of preserved preparations for *Pediculus humanus capitis*, it was found that the results of the correct clearing time used in the clearing process are 15 minutes with two repetitions.[1], [20], [21] It is possible that with repetition three times, the carbon groups contained in the toluene solution have partially absorbed the alcohol solution contained in the body of *Pediculus humanus capitis*. The clearing time must be adjusted appropriately so that the tissue is not too hard, causing the *Pediculus humanus capitis* to become brittle and one of its morphologies to be lost.

The use of a clove oil solution (*Syzigium aromaticum*) is better than the use of a toluene solution because the use of a toluene solution has flammable properties; it contains less carbon, namely only 90.5%; toluene is also a dangerous and toxic substance.[20], [22] Indonesia has a variety of natural ingredients that can be used as natural insecticides. Natural ingredients that have the potential to treat *pediculus humanus capitis* infections, one of which is Clove Oil (*Syzigium aromaticum*)[18]. In comparison, toluene, with the chemical formula C<sub>7</sub>H<sub>8</sub>, is a flammable and toxic liquid derived from petroleum, commonly used as a solvent in industrial applications. It poses fire hazards and health risks upon exposure. On the other hand, clove oil, primarily composed of eugenol, is derived from the clove plant (*Syzygium aromaticum*). It boasts antimicrobial properties and is considered safer for use due to its natural composition. Unlike toluene, clove oil is not flammable and is often utilized in traditional medicine and aromatherapy. This comparison underscores the potential advantages of clove oil, particularly in terms of safety and natural properties [23].

In this study, clove oil (*Syzigium aromaticum*) proved to be a superior clearing agent compared to toluene, as evidenced by the enhanced quality of tissue preparations. This finding aligns with prior research highlighting the efficacy of clove oil due to its high eugenol content (95%), which facilitates the removal of alcohol residues during the clearing process[24]. Moreover, earlier studies have indicated variations in optimal clearing times depending on the clearing agent utilized, with toluene requiring 5 minutes and clove oil and xylol necessitating 10 minutes for optimal results [25].

The theoretical implication of these findings lies in confirming the potential of clove oil as a safe and effective alternative to toluene for tissue clearing purposes. The presence of eugenol in clove oil contributes to improved clarity and quality of tissue preparations by minimizing alcohol residues. Practically, the use of clove oil offers a safer option, particularly in regions like Indonesia, where natural resources are abundant and the potential for natural alternatives to synthetic chemicals is high [25].

## **SIMPULAN**

Based on the Mann-Whitney test, it can be concluded that there was a difference between the use of toluene solution and clove oil (*Syzigium aromaticum*) in the clearing process on the quality of permanent preparations (good, quite good and bad), and the use of oil cloves (*Syzigium aromaticum*) is better than using toluene. to replace xylol (xylene) as a clearing agent. Further research is recommended to use a clove oil (*Syzigium aromaticum*) solution, which will be compared with other solutions and use

different soaking times for different stages. One such limitation lies in the sample size and its potential impact on the generalizability of the findings. The study may have utilized a limited sample, thereby restricting the direct application of its results to a broader population. Utilizing a larger sample size and incorporating greater variation in research parameters could enhance the generalizability of the findings.

## REFERENCES

- [1] F. Septiani, "Perbedaan Kualitas Preparat Permanen Pediculus Humanus Capitis Pada Proses Clearing Menggunakan Xylol Dan Minyak Cengkeh." Universitas Muhammadiyah Semarang, 2018.
- [2] F. Azim and N. Andrini, "Perbandingan Angka Kejadian Pedikulosis Kapitis Antara Anak Laki-Laki Dengan Anak Perempuan di Pondok Pesantren Al-Kautsar Al-Akbar Medan," *J. Ibnu Sina Biomedika*, vol. 2, no. 1, pp. 72–79, 2018.
- [3] A. Iswara and F. Nuroini, "Variasi Konsentrasi Koh Dan Waktu Clearing Terhadap Kualitas Preparat Awetan Pediculus humanus capitis," in *Prosiding Seminar Nasional & Internasional*, 2017, vol. 1, no. 1.
- [4] G. Purnasari, D. Briawan, and C. Dwiriani, "Asupan Kalsium Dan Tingkat Kecukupan Kalsium Pada Ibu Hamil Di Kabupaten Jember," *Media Kesehat. Masy. Indones. Univ. Hasanuddin*, vol. 12, no. 4, pp. 261–268, 2016.
- [5] B. Nurhayati, S. Darmawati, and P. P. S. D. M. Kesehatan, "Bahan Ajar Teknologi Laboratorium Medis (TLM): Biologi Sel dan Molekuler." Pusat Pendidikan SDM Kesehatan Badan PPSDM Kesehatan, Kemenkes RI, 2018.
- [6] L. R. Hasanah, "Pengaruh Pemberian Filtrat Buah Murbei (*Morus alba* L) sebagai Pewarna Alternatif terhadap Kualitas Preparat Gosok Tulang Femur Kelinci (*Oryctolagus cuniculus* L)." Universitas Muhammadiyah Malang, 2019.
- [7] A. Damayanti and S. Bariroh, "PENGOLAHAN BIJI MAHONI (*Swietenia Macrophylla* King) sebagai Bahan Baku Alternatif Biodiesel," *J. Bahan Alam Terbarukan*, vol. 1, pp. 9–12, 2012, [Online]. Available: <https://journal.unnes.ac.id/nju/index.php/jbat/article/view/2539/2592>.
- [8] R. Kandyala, S. P. C. Raghavendra, and S. T. Rajasekharan, "Xylene: An overview of its health hazards and preventive measures.," *J. Oral Maxillofac. Pathol.*, vol. 14, no. 1, pp. 1–5, Jan. 2010, doi: 10.4103/0973-029X.64299.
- [9] J. M. Clark, "New chemistries for the control of human head lice, *Pediculus humanus capitis*: A mini-review," *Pestic. Biochem. Physiol.*, vol. 181, p. 105013, 2022.
- [10] J. N. Haro-González, G. A. Castillo-Herrera, M. Martínez-Velázquez, and H. Espinosa-Andrews, "Clove essential oil (*Syzygium aromaticum* l. myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health," *Molecules*, vol. 26, no. 21, 2021, doi: 10.3390/molecules26216387.
- [11] J. K. Straughen *et al.*, "The Impact of Environmental Benzene, Toluene, Ethylbenzene, and Xylene Exposure on Blood-Based DNA Methylation Profiles in Pregnant African American Women from Detroit," *Int. J. Environ. Res. Public Health*, vol. 21, no. 3, 2024, doi: 10.3390/ijerph21030256.
- [12] R. Tsamiya *et al.*, "Comparative Evaluation of Clove, Olive and Groundnut Oil's Clearing Ability in Tissue Processing," *J. Med. Lab. Sci.*, vol. 31, pp. 43–53, Mar. 2021, doi: 10.5281/zenodo.4641412.
- [13] N. Azizah, E. Y. Mahtuti, and Faisal, "Fixation Process With 10% KOH Immersion And Variation Of Heating Temperatures On The Quality Of *Pediculus humanus capitis*," *Medicra (Journal Med. Lab. Sci.)*, vol. 5, no. 2, pp. 80–85, 2022, doi: 10.21070/medicra.v5i2.1635.
- [14] A. Damayanti, B. Triwibowo, M. Megawati, A. T. Ekanuramanta, H. Harianingsih, and K. Thomas, "Optimization of The Aqueous Enzymatic Extraction (AEE) of Rice Bran Oil With Cellulase Using Response Surface Methodology," *J. Bahan Alam Terbarukan*,

- vol. 12, no. 1, pp. 87–96, 2023.
- [15] B. Sadanandan *et al.*, “Aqueous spice extracts as alternative antimycotics to control highly drug resistant extensive biofilm forming clinical isolates of *Candida albicans*,” *PLoS One*, vol. 18, no. 6, p. e0281035, 2023.
- [16] A. Ghofur, T. Suparyati, and A. Qolbi, “Pengaruh Variasi Waktu Clearing (Penjernihan) Toluene Terhadap Kualitas Sediaan Permanen *Cimex lectularis*,” *J. Med. Husada*, vol. 2, no. 1, pp. 29–34, 2022.
- [17] I. P. D. K. Yadnya, F. Azmi, A. Andriana, and A. V. W. Taufiq, “Efektivitas Kombinasi Ekstrak Daun Jeruk Nipis dan Perasan Buah Jeruk Nipis (*Citrus aurantifolia*) terhadap Mortalitas Kutu Rambut (*Pediculus humanus var capitis*),” *Nusant. Hasana J.*, vol. 2, no. 11, pp. 101–111, 2023.
- [18] R. Shalsadila, M. Nuryanti, and P. Purwaeni, “Review Artikel: Potensi Berbagai Bahan Alam Sebagai Insektisida Alami Kutu Rambut (*Pediculus humanus capitis*),” *J. Pharm. Sci.*, vol. 6, no. 2, pp. 664–672, 2023.
- [19] P. Bauer and A. Buettner, “Quantification of odorous and potentially harmful substances in acrylic paint,” *Ecotoxicol. Environ. Saf.*, vol. 262, p. 115329, 2023.
- [20] A. Putri, Y. S. Mulia, S. Sulaeman, and W. Wiryanti, “Pengaruh Variasi Waktu Perendaman dengan Alkohol terhadap Kualitas Preparat Permanen Larva *Culex sp.*,” *J. Kesehat. Siliwangi*, vol. 4, no. 1, pp. 340–345, 2023.
- [21] S. Aisyah, E. Y. Mahtuti, M. Masyhur, and F. Faisal, “Perbandingan Penggunaan Pelarut Organik Xilene Dengan Toluena Pada Tahapan Clearing Terhadap Kualitas Preparat Aetan *Aedes Albopictus* (*Stegomyia albopictus*),” *Anakes J. Ilm. Anal. Kesehat.*, vol. 9, no. 1, pp. 20–27, 2023.
- [22] E. Abbasi *et al.*, “Evaluation of resistance of human head lice to pyrethroid insecticides: a meta-analysis study,” *Heliyon*, vol. 9, no. 6, 2023.
- [23] N. C. for B. Information, “PubChem Compound Summary for CID 1140, Toluene,” *National Center for Biotechnology Information*, 2024. .
- [24] M. A. Muwaffaq and E. Supriyo, “Optimization of clove flower oil extraction (*Syzygium aromaticum* L.) With Factorial Design Method,” *J. Vocat. Stud. Appl. Res.*, vol. 3, no. 2, pp. 38–41, 2021, doi: 10.14710/jvsar.v3i2.12182.
- [25] N. Alwahaibi, S. Aljaradi, and H. Alazri, “Alternative to xylene as a clearing agent in histopathology,” *J. Lab. Physicians*, vol. 10, no. 2, pp. 189–193, 2018, doi: 10.4103/JLP.JLP\_111\_17.