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Analysis of the Use of Virgin Coconut Oil as an Alternative Soaking Agent on the Quality of *Ctenocephalides felis* Preparations

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Abstrak

Preservation preparations are a technique for preserving parasite specimens, including *Ctenocephalides felis* (cat flea), involving several stages: fixation with 10% KOH, dehydration using graded alcohols (30%, 50%, 70%, 96%, absolute), clearing, and mounting. The clearing process aims to clarify the morphological structure of the parasite. This study aims to investigate the function of virgin coconut oil (VCO) as an alternative to xylene in the clearing stage, in terms of the clarity and quality of the preparation. The research design used is a quasi-experimental approach with purposive sampling technique. The research population consists of fleas, and the sample included 16 cat fleas (*Ctenocephalides felis*) that underwent the clearing process using virgin coconut oil (VCO). The results of the study showed that out of the 16 preparations, 9 still had residual chitin layers, while 7 did not. Based on the quality of the preparation, there were 7 preparations with good quality (44%), 6 moderately good (38%), and 3 poor (18%). This study concluded that virgin coconut oil is capable of clarifying *Ctenocephalides felis* preservation preparations and can serve as an alternative to xylene in the clearing process, with the preparation quality being sufficient for microscopic morphological observation of fleas.

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1. INTRODUCTION

Fleas are ectoparasites that live on the surface of their host's body. Often, people cannot distinguish between lice and fleas. Systematically, fleas belong to the phylum Arthropoda, class Insecta, and order Siphonaptera. There are several important genera in this family, such as Tungau (chigoe flea), Ctenocephalides (cat and dog fleas), Echidnophaga (chicken flea), Pulex, Ceratophyllus, and Xenopsylla (rat flea) (Adrianto, Berdman, & Arwati, 2022). Ctenocephalides felis, or cat fleas, can disturb humans and animals both directly and indirectly. Directly, they can cause a specific skin reaction known as flea allergic dermatitis. In addition to direct disturbances, fleas also play a role in transmitting several diseases that are harmful to both humans and animals, such as tapeworm infestations, where Ctenocephalides felis can serve as a host for Dipylidium caninum. This tapeworm can infect Ctenocephalides felis in its larval stage when it feeds on organic material around its host, which can include tapeworm eggs from infected cat feces (Sigit et al., 2006).

The exoskeleton is the hard outer structure made of chitin, proteins, and various minerals. This exoskeleton serves as protection from external environments and gives the flea its form and body structure. In arthropods, the exoskeleton is mainly composed of the cuticle, which is an organic substance called chitin. Chitin naturally bonds with proteins and minerals. There are two methods for degrading chitin: enzymatic and chemical methods. Enzyme proteases and lactic acid fermentation are used in the enzymatic method, while acids and bases are used in the chemical method. Chemicals tested for deproteinization include NaOH, Na₂CO₃, NaHCO₃, KOH, K₂C₂O₃, K₂CO₃, Ca(OH)₂, Na₂SO₃, CaHSO₃, Na₃PO₄, and Na₂S (Afriani et al., 2016).

To understand the morphology and identify parasites affecting humans, preservation preparations are made by expert Medical Laboratory Technologists. The preparation process involves several stages, and errors can occur during these stages. Mistakes in preparing the specimen can result in damage, such as unclear visibility, blurred body parts, incomplete specimens, or missing parts of the flea's body, and the specimen may not last for long periods (Widiyanti, 2013).

The first stage in preparing a preserved Ctenocephalides felis (cat flea) specimen involves fixation by soaking in KOH (Sodium Hydroxide), which acts as a deproteinizing agent due to its effectiveness in breaking the bond between proteins and chitin. This is due to the strong alkaline nature of KOH, which activates the hydrogen bonds between proteins and chitin, allowing the protein to dissolve in the KOH solution. As a result, the dark color of the flea's body fades due to thinning of the exoskeleton and its structural components (Azizah et al., 2022). After fixation, dehydration is performed using graded alcohol to gradually reduce the water content in the sample. This dehydration process works through osmosis. Alcohol has a stronger attraction to water than the flea's tissue, and when the flea is soaked in graded alcohol (30%, 50%, 70%, 96%, and absolute), it progressively replaces the water in the sample with alcohol, with absolute alcohol being used to remove any remaining water (Sari et al., 2015).

Next, the clearing process is done using clearing solutions such as xylene, toluene, and benzene. The purpose of clearing is to make the specimen transparent and replace the alcohol in the tissue. Xylene or dimethylbenzene (C₆H₄(CH₃)₂) is commonly used for this purpose (Iswara & Nuroini, 2017). The clearing agent removes substances that obscure or block microscopic views of the specimen, such as residual fats.

Xylene is commonly used as a solvent to replace alcohol during the clearing stage. Xylene has the ability to dissolve alcohol, and after the alcohol is dissolved, it fills the space previously occupied by alcohol. Xylene is also used to remove fats, enhancing the transparency of the specimen, making tissue structures more visible under a microscope

(Ariyadi, 2022). However, xylene has several drawbacks. Prolonged immersion in xylene can make specimens brittle and dry. Additionally, it poses health risks to laboratory technicians, including respiratory irritation, damage to the central nervous system, kidney and liver damage, and toxicity and carcinogenicity (United States Department of Health and Human Services, 2017). To protect health workers from xylene exposure, alternatives are being explored.

One alternative is Virgin Coconut Oil (VCO), which has shown promise in research by Hakim et al. (2020). VCO contains several fatty acids, including caproic acid (0.187%), octanoic acid (1.12%), cyclopropanepentanoic acid (0.54%), lauric acid (32.73%), myristic acid (28.55%), palmitic acid (17.16%), oleic acid (14.09%), and stearic acid (5.68%). These compounds are suspected to have potential for dissolving alcohol due to their non-polar properties, allowing the oil to dissolve organic compounds. The fatty acid content in coconut oil makes it a suitable substitute for xylene in the clearing stage.

Among the fatty acids in VCO, octanoic acid (C_8) has properties that make it effective as a solvent for fats. The structure of fatty acids with varying carbon chain lengths influences their solubility and non-polar characteristics. Longer carbon chains, such as oleic acid (18 carbons), enhance fat dissolution. Oleic acid has both polar and non-polar properties, allowing it to interact with water and fats. The non-polar hydrocarbon chain helps break down fats into smaller particles, making them easier to remove (Nitbani et al., 2022).

Studies by Sofyanita & Azahra (2023) showed that VCO could be used as a clearing solution for liver specimens in mice. Their research found that 100% of the preparations were categorized as good based on transparency and texture. Similarly, research by Fitriana & Fitri (2020) highlighted the effectiveness of coconut oil, particularly its lauric acid content, which is similar to xylene in its ability to dissolve fats. The results suggest that coconut oil can serve as a viable alternative to xylene in the clearing process. This study aims to evaluate the function of Virgin Coconut Oil (VCO) as an alternative to xylene in the clearing stage, based on the clarity and quality of the preparation. The findings of this study could lead to safer and more environmentally friendly alternatives for preparing preserved parasite specimens, offering a viable solution to the drawbacks associated with traditional chemicals like xylene.

2. METHOD

This study uses a Quasi Experimental Design, with fleas as the research population. The sample used in this study is cat fleas (*Ctenocephalides felis*), which were selected using purposive sampling technique. The type of data used in this study is primary data, specifically related to the effect of Virgin Coconut Oil (VCO) on the quality of cat flea (*Ctenocephalides felis*) preparations. This data was then analyzed using univariate analysis.

In univariate analysis, the data collected from the study was presented in the form of a distribution table. This method helps to organize the data into understandable categories, allowing for a clear overview of the sample and the variables being examined. The distribution table is useful in summarizing the characteristics of the sample, and it helps to highlight key patterns or trends that may emerge from the data.

The data collected from the study was processed and analyzed descriptively. Descriptive analysis aims to provide an overview of the data, summarizing it without making inferences or drawing conclusions beyond the data itself. This allows the researcher to interpret the results in a meaningful way, providing insights into the effects of Virgin Coconut Oil on the quality of flea preparations and making it easier to communicate the findings of the study to others.

3. RESULTS AND DISCUSSION

Evaluation based on the research conducted on cat flea samples during the Clearing stage is as follows:

Table 1. Results of Virgin Coconut Oil Function as an Alternative to Xylene in the Clearing Process for Chitin Layer Residue in Preserved *Ctenocephalides felis* Preparations

Treatment	Chitin Layer Residue		Total
	Present	Absent	
Virgin Coconut Oil (VCO)	9	7	16

Table 1 shows that in the Clearing treatment using Virgin Coconut Oil (VCO), 9 preparations still had chitin residue, meaning the preparations were clear.

Table 2. Quality of *Ctenocephalides felis* Preserved Preparations with the Clearing Process Using Virgin Coconut Oil (VCO).

Treatment	Chitin Layer Residue			Total
	Poor	Moderate	Good	
Virgin Coconut Oil (VCO)	3	6	7	16
	18%	38%	44%	100%

Table 2 shows that in the Clearing treatment using Virgin Coconut Oil (VCO), 3 preparations had poor quality, meaning under the microscope, they were unclear, had chitin residue $> \frac{1}{4}$, and the morphology was incomplete, with a percentage of 18%. Next, 6 preparations had fair quality, meaning they were clear under the microscope, had chitin residue $< \frac{1}{4}$, and the morphology was incomplete, with a percentage of 38%. There were 7 preparations with good quality, meaning they were clear, had no chitin residue, and the morphology was intact, with a percentage of 44%.

DISCUSSION

This study was conducted with the Clearing process using cat flea samples (*Ctenocephalides felis*) that were clarified using Virgin Coconut Oil (VCO) as an alternative to xylene for preserved preparations with good quality. The preparations with good quality are those that, when observed under a microscope, appear clear, with no chitin residue and intact morphology. Fair quality preparations are those that, when observed under a microscope, appear clear or have chitin residue $< \frac{1}{4}$, and the morphology is not intact. Poor quality preparations are those that, when observed under a microscope, do not appear clear, have chitin residue $> \frac{1}{4}$, and the morphology is not intact. Based on the results of the study, it was found that Virgin Coconut Oil (VCO) is capable of clarifying cat fleas (*Ctenocephalides felis*), as seen from the preserved preparations that were cleared using VCO, which appeared clear under the microscope. Table 1 shows that the Clearing process using Virgin Coconut Oil resulted in 9 preparations that still had chitin residue, indicating that the preparations were not clear. This was influenced by the thickness of the flea's exoskeleton, making it difficult for the Virgin Coconut Oil to penetrate the thicker skin layers during the Clearing process.

According to Adrianto et al. (2022), adult fleas have a thicker and denser exoskeleton. For preparations of good quality with standard xylene use, the preparations appear clear, with complete morphology, with no broken, damaged, or missing parts. Research by Lael et al. (2018) indicates that xylene is more effective in clarifying preserved preparations than Virgin Coconut Oil (VCO) because xylene has a chemical composition that contains more hydrocarbon compounds, which have clarifying properties. Then, for the 7 preparations with no chitin residue, they appeared clear. These 7 preparations were likely from younger fleas, as evidenced by their lighter brown skin color,

which means they had thinner exoskeletons, allowing the components of Virgin Coconut Oil, such as oleic acid, to interact, making the preparations appear clear. Based on the preparation quality results in Table 2, 3 preparations were of poor quality, with a percentage of 18%. Poor quality preparations are those that do not appear clear, have chitin residue, and the morphology is not intact. These 3 poor-quality preparations were all adult fleas, which have a denser exoskeleton and darker body color.

According to Sigit et al., (2006), when fleas are still young, their body structure is not fully formed. As they age, their exoskeleton becomes thicker and more complex, with a more solid structure and deeper pigmentation. As fleas age, Virgin Coconut Oil (VCO) has difficulty penetrating their exoskeleton, and it is less effective in replacing the remaining absolute alcohol, as it does not penetrate the flea's body perfectly because it still contains alcohol. Therefore, the preservation quality is not always the same as when using xylene for Clearing. Fairly good quality was obtained for 6 preparations with a percentage of 38%, and 7 preparations were of good quality with a percentage of 44%. For the preparations with fair and good quality, when observed under a microscope, they appeared clear. However, for fair-quality preparations, the morphology was not intact, while for good-quality preparations, the morphology was intact. This shows that Virgin Coconut Oil (VCO), which contains fatty acids such as lauric acid, myristic acid, palmitic acid, and oleic acid, can interact perfectly after the dehydration stage, where oleic acid can interact with alcohol. In the Clearing stage, Virgin Coconut Oil is able to penetrate all structures of the flea, making the preparations appear clear under the microscope. For the 6 fairly good preparations, incomplete morphology was found due to the presence of young fleas, whose body structure is more easily detached during sample collection from the cat's body when the tools, such as metal tweezers, are not properly used.

4. CONCLUSION

The results of the study showed that from the preserved preparations using Virgin Coconut Oil (VCO), 9 preparations still contained chitin residue, making them appear unclear when observed under the microscope. Meanwhile, 7 preparations did not show any chitin residue, so they appeared clear microscopically. Based on the quality of the preparations, the results showed that 3 preparations were categorized as poor quality with a percentage of 18%. These preparations appeared unclear under the microscope, with chitin residue more than a quarter of the structure and incomplete morphology. There were 6 preparations with fairly good quality, accounting for 38%, where the preparations appeared clear but still had less than a quarter of chitin residue, and the morphology was not intact. Meanwhile, 7 preparations were categorized as good quality with a percentage of 44%, as they appeared clear under the microscope, had no chitin residue, and their morphology was intact.

REFERENCES

- Adrianto, H., Subekti, S.S., Arwati, H., Indah S., T., Mellynawati,... & Mitak, C. (2022). *Entomologi Untuk Kedokteran Dan Kesehatan*. Yogyakarta: Rapha Publishing.
- Afriani, Y., Fadli, A., Maulana, S., & Karina, I. (2016). Sintesis , Kinetika Reaksi dan Aplikasi Kitin dari Cangkang Udang : Review. *Seminar Nasional Teknik Kimia – Teknologi Oleo Petro Kimia Indonesia*, 184-196.
- Ariyadi, T. (2022). Proses Deparafinasi Sediaan Jaringan Ginjal Dengan Dan Tanpa Pemanasan Menggunakan Mineral Oil Pada Pewarnaan Hematoksilin-Eosin. *Jurnal Kesehatan Rajawali*, 11(2), 1–6. <https://doi.org/10.54350/jkr.v11i2.104>
- Azizah, N., & Mahtuti, E. Y. (2022). Fixation Process With 10% KOH Immersion And Variation Of Heating Temperatures On The Quality Of *Pediculus Humanus Capitis*. *Medicra (Journal of Medical Laboratory Science/Technology)*, 5(2), 80-85.

<https://doi.org/10.21070/medicra.v5i2.1635>

- Fitriana, Y. A. N., & Fitri, A. S. (2020). Uji Lipid pada Minyak Kelapa, Margarin, dan Gliserol. *Sainteks*, 16(1), 19–23. <https://doi.org/10.30595/sainteks.v16i1.7013>
- Hakim, M. Z. F., Handayani, W. A. F., Fauziah, S. N., & Haryanto, H. (2020). Kajian : Karakter , Proses dan Potensi *Virgin Coconut Oil* (VCO) Sebagai Pangan Fungsional. *Journal of Science, Technology, and Entrepreneurship*, 2(2), 33–39.
- Iswara, A., & Nuroini, F. (2017). Variasi Konsentrasi KOH Dan Waktu *Clearing* Terhadap Kualitas Prerapat Awetan *Pediculus humanus capitis*. *Prosiding Seminar Nasional Publikasih Hasi-Hasil Penelitian Dan Pengabdian Masyarakat*, 3(1), 60–63.
- Lael, B. F., Santosa, B., & Aryadi, T. (2018). Perbedaan Penggunaan *Xylol* (*Xylene*) dan *Toluol* (*Toluene*) pada Proses *Clearing* terhadap Kualitas Preparat Awetan Permanen *Cimex lectularius*. *Prosiding Seminar Nasional Mahasiswa Unimus*, 1(3), 232–237.
- Nitbani, F. O., Jumina, Siswanta, D., & Solikhah, E. N. (2016). Isolation and Antibacterial Activity Test of Lauric Acid from Crude Coconut Oil (*Cocos nucifera* L.). *Procedia Chemistry*, 18(February), 132–140. <https://doi.org/10.1016/j.proche.2016.01.021>
- Sari, Dewi, P., Umi, F., & Rizky, Maya, P. (2015). *Profil Hands on Activity* pada Mata Kuliah Mikroteknik di Prodi Pendidikan Biologi FKIP UNS. *Proceeding Biology Education Conference* 13(1): 476-481.
- Sigit, H. S., Koesharto, F., Hadi, U.K., Gunandini, S.J., Soviana, S., Wirawan, I.A., ... & Utomo, S. (2006). *Hama Perbukitan Indonesia: Pengenalan, Biologi dan Pengendalian*. Bogor: Unit Kajian Pengendalian Hama Perbukitan. Unit Kajian Pengendalian Hama Perbukitan, Fakultas Kedokteran Hewan, Institut Pertanian Bogor.
- Sofyanita, E. N., & Azahra, N. (2023). Pengaruh Penggunaan Minyak Kelapa Murni Sebagai Larutan *Clearing* Pada Sediaan Hepar Mencit. *Jurnal Analis Laboratorium Medik*, 8(1), 57–65. <https://doi.org/10.51544/jalm.v8i1.3945>
- United States Department of Health and Human Services. (2017). *2017 Department of Health and Human Services (HHS) Annual Computer Matching Report*. United States Department of Health and Human Services.
- Widiyanti, N. L. P. M. (2013). Pola Perindukan Nyamuk Yang Ditangkap di Perindukan di Kabupaten Buleleng dan Manfaatnya Sebagai Bahan Praktikum Dalam Perkuliahan Zoologi Invertebrata. *Jurnal IKA*, 11(1), 27–41.