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Head Lice Mortality Activity (*Pediculus humanus capitis*) After Treating with Combination of Soursop Leaves Extract and Eucalyptus Oil

(AKTIVITAS MORTALITAS KUTU KEPALA (*PEDICULUS HUMANUS CAPITIS*) SETELAH DIOBATI DENGAN KOMBINASI EKSTRAK DAUN SIRSAK DAN MINYAK KAYU PUTIH)

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ABSTRACT

Pediculosis capitis is a scalp infection caused by the obligate ectoparasite *Pediculus humanus capitis*. This condition can be treated through various methods, including the use of medications. Soursop leaf extract and eucalyptus oil are known to exhibit lethal activity against *P.h. capitis* due to the presence of acetogenins, isoflavones, and cineole. This study was aimed to investigate the mortality activity of *P.h. capitis* following treatment with a combination of soursop leaf extract and eucalyptus oil. The study employed a true experimental design, specifically a post-test-only control group design, comprising two control groups (1% permethrin as the positive control (K+) and distilled water as the negative control (K-)) and five treatment groups with combinations of 5% soursop leaf extract and 5% eucalyptus oil (P1=1:1, P2=1:2, P3=2:1, P4=1:3, P5=3:1). The effectiveness of the combinations on *P.h. capitis* mortality was analyzed using *Friedman*, while the LC_{50} and LT_{50} values were determined through probit analysis. The results showed significant differences in mortality rates between the negative control group and the positive control group, as well as all treatment groups. Significant differences were also observed between the positive control group and the treatment groups P3, P4, and P5. However, no significant differences were found among the treatment groups at various observation times. The treatment results of group P2 (1:2) were the most effective in killing *P. h. capitis*. The combination of soursop leaf extract and eucalyptus oil has been proven effective as a natural pediculicide..

Keywords: *Pediculus humanus capitis;* Eucalyptus; herbal; pediculicide; soursop

ABSTRAK

Pedikulosis kapitis adalah infeksi kulit kepala yang disebabkan oleh ektoparasit obligat *Pediculus humanus capitis.* Penyakit ini dapat disembuhkan menggunakan beberapa cara, salah satunya dengan pemberian obat. Ekstrak daun sirsak dan minyak kayu putih diketahui memiliki daya bunuh terhadap *P.h. capitis* karena mengandung acetogenin, isoflavon, dan sineol. Penelitian ini bertujuan untuk mengetahui aktivitas mortalitas *P.h. capitis* dari pemberian kombinasi daun sirsak dan minyak kayu putih. Penelitian ini berjenis *true experimental* dengan desain penelitian post test only with control group design yang terdiri atas dua kelompok kontrol (permetrin 1% (K+) dan aquades (K-)) dan lima kelompok perlakuan kombinasi ekstrak daun sirsak 5% dan minyak kayu putih 5% (P1 = 1:1, P2 = 1:2, P3= 2:1, P4= 1:3, P5= 3:1). Data efektivitas kombinasi bahan terhadap mortalitas *P.h. capitis* dianalisis dengan uji *Friedman*, sedangkan data nilai LC_{50} dan LT_{50} dianalisis menggunakan analisis probit. Terdapat perbedaan mortalitas antara kelompok kontrol negatif dengan kontrol positif dan seluruh kelompok perlakukan. Perbedaan kelompok kontrol positif dengan kelompok perlakuan P3, P4 dan P5, namun tidak terdapat perbedaan antara kelompok perlakukan pada setiap waktu pengamatan. Hasil perlakuan kelompok P2 (1:2) paling efektif dalam membunuh *P.h. capitis*. Kombinasi ekstrak daun sirsak dan minyak kayu putih terbukti efektif sebagai pedikulosida alami.

Kata-kata kunci: *Pediculus humanus capitis*; eucalyptus; herbal; pedikulosida; sirsak

INTRODUCTION

Pediculosis capitis is a scalp infection caused by the infection of *Pediculus humanus capitis*, an obligate ectoparasite that survives by feeding on blood from the scalp. The bites of *P.h. capitis*, which feeds approxymately every 3–6 hours, often causes a range of complications, including itching, skin irritation and sores (Leung *et al*., 2022). This condition is particularly prevalent among school-aged children in both developed and developing country. The highest prevalence rates of Pediculosis capitis have been recorded in Libya (78.6%), Turkiye (69.5%), Malaysia (35%), and Thailand (23.48%) (Lintong *et al*., 2021). While the exact prevalence in Indo-nesia remains uncertain, reports indicate rates as high as 88.6% in Central Kalimantan and 88.2% in Riau Province (Trasia, 2023).

Pediculosis capitis is often perceived as a minor health issue, leading to its classification as a Neglected Tropical Disease. However, emerging research indicates that *P.h. capitis* can serve as a vector for various pathogenic microorganisms such as *Acinetobacter baumannii* (Arrizqiyani *et al*., 2019; Mokhtar *et al*., 2020). Additionally, pediculosis can trigger dermatological complications, including irritation, allergic reactions, and open wounds, which may significantly impact an individual's self-esteem and overall psychological well-being (Nurmatialila *et al*., 2019).

Control of *P.h. capitis* infection has traditionally relied on various synthetic pediculocidal agents, including permethrin, dichloro diphenyl trichloroethane (DDT), pyrethrins and lindane (Husni and Al-Waiz, 2021). While these compounds have demonstrated significant efficacy in increasing the mortality of *P.h. capitis*, their prolonged use poses significant risks, such as the development of clinical resistance in the parasite. Among these agents, lindane has been associated with severe adverse effects in humans, including neurological complications. Reported side effects of lindane include severe skin irritation, dizzyness, headaches, diarrhea, nausea, vomiting, seizures and in extreme cases, fatalities (Arrizqiyani *et al*., 2019).

The adverse effects associated with synthetic pediculocides can be mitigated by utilizing natural alternatives, such as garlic, soursop leaves, lime and fragrant pandanus leaves (Shalsadila *et al*., 2023). Natural ingredients offer several advantages: they are environmentally friendly, biodegradable and generally safer for users (Aripin *et al*., 2022). Among these, soursop leaves and eucalyptus oil have demonstrated significant potential as alternative pediculocides for managing Pediculosis capitis. Soursop leaves contain acetogenin, which functions as both an antifeedant and stomach toxin for *P.h. capitis*, and isoflavones, which disrupt the insect's reproductive system. Research indicates that a soursop leaf extract shampoo can achieve 100% mortality of *P.h. capitis* within an average time of 11 seconds (Samosir *et al*., 2023). Similarly, soursop seed extract at concentrations of 80% and 100% can effectively eliminate *P.h. capitis* within 10 minutes (Muflihah and Sasongkowati, 2021).

Eucalyptus oil, on the other hand, contains cineole, a compound that acts as an effective toxin for *P.h. capitis* (Shalsadila *et al*., 2023). A combination of 75% eucalyptus oil and 25% lime has been shown to kill 100% of *P.h. capitis* within just five minutes (Aripin *et al*., 2022). Furthermore, shampoo formulations with 20% eucalyptus oil concentration are capable of achieving 100% mortality of *P.h. capitis* within an average time of 24.7 minutes (Dalming *et al*., 2022). These findings highlight the efficacy and environmental compatibility of natural alternatives in addressing pediculosis.

The potential of combining soursop leaf extract with eucalyptus oil as a treatment for *P.h. capitis* mortality remains underexplored. The objective of this study was to assess the efficacy of this combination in inducing mortality in *P.h. capitis*, providing valuable insights into its potential as a natural pediculocidal alternative.

RESEARCH METHODS

This study employed a true experimental design with a post-test-only control group structure. The experiment included two control groups, positive control (1% permethrin, K+) and negative control (distilled water, K-) and five treatment groups receiving a combination of 5% soursop leaf extract and 5% eucalyptus oil in varying ratios. The ratio of natural ingredients selected for use as a pediculicidal agent was adopted from the study conducted by Aripin *et al*. (2022).

Group P1: Combination of soursop leaf extract and eucalyptus oil in a 1:1 ratio.
Group P2: Combination of soursop leaf extract and eucalyptus oil in a 1:2 ratio.
Group P3: Combination of soursop leaf extract and eucalyptus oil in a 2:1 ratio.
Group P4: Combination of soursop leaf extract and eucalyptus oil in a 1:3 ratio.
Group P5: Combination of soursop leaf extract and eucalyptus oil in a 3:1 ratio.

Each groups comprised 10 adult *P.h. capitis* and the experiments were repeated three times for accuracy. Specimens were collected from individuals with pediculosis using a standard hair combing technique with a fine-toothed lice comb (Hayati and Ayuputri, 2021). Samples were selected using an random sampling method and mortality of *P.h. capitis* was observed at 5-minute intervals over the course of one hour.

This study was conducted between May and June 2024, following ethical approval from the Health Research Ethics Commission of Muhammadiyah Purwokerto University (registration number: KEPK/UMP/83/V/2024). Sample identification and determination were performed at the Pharmaceutical Biology Laboratory within the Faculty of Pharmacy, Muhammadiyah Purwokerto University. The preparation of the soursop leaf extract was carried out at the Polymer Hydrogel Laboratory, Faculty of Engineering and Science, Muhammadiyah Purwokerto University, while the assessment of *P.h. capitis* mortality after treatment with the combination of soursop leaf extract and eucalyptus oil was conducted at the Medical Laboratory Technology Laboratory, Faculty of Health Sciences, Universitas Muhammadiyah Purwokerto.

Tools and Materials

The equipment utilized in this study included a dropper pipette, beaker, spatula, measuring cup, lice comb, vortex mixer, test tubes, sieve, blender, stopwatch, evaporator, bag or cardboard box, and plastic container. The materials employed comprised distilled water (aquadest), soursop leaves, eucalyptus oil, filter paper, 1% permethrin, ethanol and tissue. *P.h. capitis* samples were initially examined under a microscope to ensure the selection of adult specimens for the treatment. Adult *P. h. capitis* were recognized by their dark pigmentation, segmented antennae and specialized mouthparts adapted for sucking. Males possessed an aedagus, whereas females displayed a genital pore (Susanty *et al*., 2020).
Preparation of Soursop Leaf Extract

Soursop leaves were thoroughly cleaned and cut into small pieces before being dried. Once fully dried, the leaves were ground into a fine powder using a blender and subsequently sieved to achieve a uniform texture. The powdered soursop leaves were then dissolved in ethanol and left to macerate for 3–five days in a tightly sealed container. The resulting solution was filtered to isolate the pure extract (Ninuk *et al*., 2024).

Concentration Determination and Combination Making

The eucalyptus oil utilized in this study was a commercially is a commercially packaged product (Cap Lang Minyak Kayu Putih®, PT Eagle Indo Pharma, Tangerang, Indonesia) containing 100% pure eucalyptus oil. The formulations comprised combinations of eucalyptus oil and soursop leaf extract in varying ratios, specifically: 1:1 (5 mL of 5% soursop leaf extract and 5 mL of 5% eucalyptus oil), 1:2 (5 mL of 5% soursop leaf extract and 10 mL of 5% eucalyptus oil), 2:1 (10 mL of 5% soursop leaf extract and 5 mL of 5% eucalyptus oil), 1:3 (5 mL of 5% soursop leaf extract and 15 mL of 5% eucalyptus oil), and 3:1 (15 mL of 5% soursop leaf extract and 5 mL of 5% eucalyptus oil) (Aripin *et al*., 2022).

Sample Collection of *P.h. capitis*

The study population consists of *P.h. capitis* collected from individuals with Pediculosis capitis at the Zam Zam Muhammadiyah Modern Islamic Junior Boarding School Banyumas Regency, Cen-tral Java) and the Huffadz Al-Itqoniyyah Islamic Junior Boarding School (Purba-lingga Regency, Central Java). The col-lected samples were examined micros-copically to classify them according to their developmental stages. Adult *P.h. capitis* were identified by their black coloration, paired antennae, and sucking type mouth-parts. Furthermore, male adults were characterized by the presence of an aedagus, while female adults exhibited a porus geni-talis (Almanfaluthi *et al*., 2025; Susanty *et al*., 2020).

Control Group Testing

Groups of 10 adult *P.h. capitis* were individually placed into containers lined with filter paper moistened with distilled water, serving as the negative control (K-). Mortality rates were recorded every five minutes over a 1-hour observation period. A comparative analysis was performed using 1% permethrin as the positive control (K+) (Arrizqiyani *et al*., 2019; Tee and Badia, 2019).

Treatment Group Testing

Containers lined with filter paper were prepared in advance. The prepared combinations of soursop leaf extract and eucalyptus oil, based on specified ratios, were poured into the containers. Subsequently, 10 adult *P.h. capitis* were introduced into each container. Observations of *P.h. capitis* activity were conducted every five minutes over a 1-hour period. Mortality was determined by the absence of movement and the detachment of their legs from the filter paper. The tests were performed using predetermined concentrations and controls, with each experiment repeated three times for consistency.

Data Analysis

Differences in effectiveness bet-ween treatment groups and between obser-vation times were evaluated using Repeated Measure Analysis of variance test and Post Hoc Tukey test with GraphPad Prism 10.4.0.

The Lethal Concentration 50 (LC_{50}) and Lethal Time 50 (LT_{50}) values were determined using probit analysis.

RESULTS AND DISCUSSION

The mortality rate of *P.h. capitis* reached 100% in the positive control group (K+) within five minutes (Table 1). In the treatment groups, 100% mortality was observed at 45 minutes in group P1, 30 minutes in group P2, 40 minutes in group P3 and 35 minutes in both groups P4 and P5. The mortality rate of *P.h. capitis* in Group P2 was significantly faster compared to Groups P1, P3, P4 and P5. This finding suggests that the combination of soursop leaf extract and eucalyptus oil in a 1:2 ratio exhibits higher efficacy in eliminating *P.h. capitis* within a shorter time frame. Survival on the human scalp can reach up to 10 days, whereas *P.h. capitis* survives only 1–2 days once removed from its host (Hayati and Ayuputri, 2021).

The interaction between treatment groups and observation time (Figure 1) contributed 4.022% to the total data variance (f= 1.70, p= 0.0036). This result indicates that each treatment group demonstrated different levels of effectiveness at different observation times.

The differences in effectiveness among treatment groups were statistically significant, accounting for 76.54% of the total variance (f= 355.44; p<0.0001). This indicates that the different treatments had a substantial impact on the outcomes, reflecting clear variations in effectiveness between groups.

The effectiveness data across different observation times showed a significant variation, contributing 6.753% to the total variance (F= 17.10; P= 0.0001). These results suggest that treatment effectiveness changed meaningfully over time, highlighting the influence of the observation period on outcomes.

The Lethal Concentration 50 (LC_{50}) value indicates that a 0.001% concentration of the combined soursop leaf extract and euca-lyptus oil effectively kills *P.h. capitis* within 25 minutes (Table 3). Additionally, the Lethal Time 50 (LT_{50}) value for group P5 is 2.163 minutes (Table 4), meaning this combination can eliminate half of the *P.h. capitis* population in this group within that time frame.

The combination of soursop leaf extract and eucalyptus oil at a 5% concentration demonstrated 100% efficacy in killing *P.h. capitis* within 30 minutes. This finding aligns with research by Tee and Badia (2019), which reported that a shampoo containing 5% soursop leaf extract achieved 85% mortality of *P.h. capitis* within 25 minutes. However, it contrasts with the studies by Nurhaini *et al*. (2020) and Samosir *et al*. (2023), which stated that soursop leaf extract alone could achieve 100% mortality in less than five minutes.

Dalming *et al*. (2022) reported that eucalyptus oil at a 20% concentration achieved 100% mortality of *P.h. capitis* within 24.7 minutes. Similarly, Aripin *et al*. 2022) found that a combination of 75% eucalyptus oil and 25% lime resulted in 100% mortality within just five minutes. These findings demonstrate that higher concentrations of eucalyptus oil signifi-cantly reduce the time required to kill *P.h. capitis*.

Soursop leaf extract effectively induces mortality of *P.h. capitis* due to its content of acetogenins and isoflavones, which disrupt both the digestive and reproductive systems of the lice (Shalsadila *et al*., 2023). Eucalyptus oil rich in cineole, a compound from the terpenoid class and also contributes to the insecticidal effects. Acetogenins, such as asimicun, bulatacin and squamocin, possess cytotoxic properties that target insects. These compounds disrupt the digestive system of *P.h. capitis*, reducing their blood-sucking appetite. Furthermore, acetogenins interfere with the electron transfer from β-nicotinamide adenine dinucleotide (NADH) to ubiqui-none, causing damage to the mitochondria of the lice (Samosir *et al*., 2023). Cineole acts as a

contact poison, respiratory toxin,

Table 1. Average mortality percentage of *Pediculus humanus Capitis*

Percentage (%) Mortality of *P.h. capitis*							
Minutes	Treatment						
	K+	K-	P1	P2	P3	P4	P5
5	100	0	77	73	40	67	40
10	100	0	83	73	60	73	77
15	100	0	83	93	83	93	80
20	100	0	87	93	83	93	88
25	100	0	90	97	90	97	90
30	100	0	97	100	93	97	97
35	100	0	97	100	93	100	100
40	100	0	97	100	100	100	100
45	100	0	100	100	100	100	100
50	100	0	100	100	100	100	100
55	100	0	100	100	100	100	100
60	100	0	100	100	100	100	100

Description : K+ (Permethrin 1%), K- (Distilled water), P1 = Soursop leaf extract 5% and eucalyptus oil (1:1), P2= Soursop leaf extract 5% and eucalyptus oil (1:2), P3= Soursop leaf extract 5% and eucalyptus oil (2:1), P4= Soursop leaf extract 5% and eucalyptus oil (1:3), P5= Soursop leaf extract 5% and eucalyptus oil (3:1).

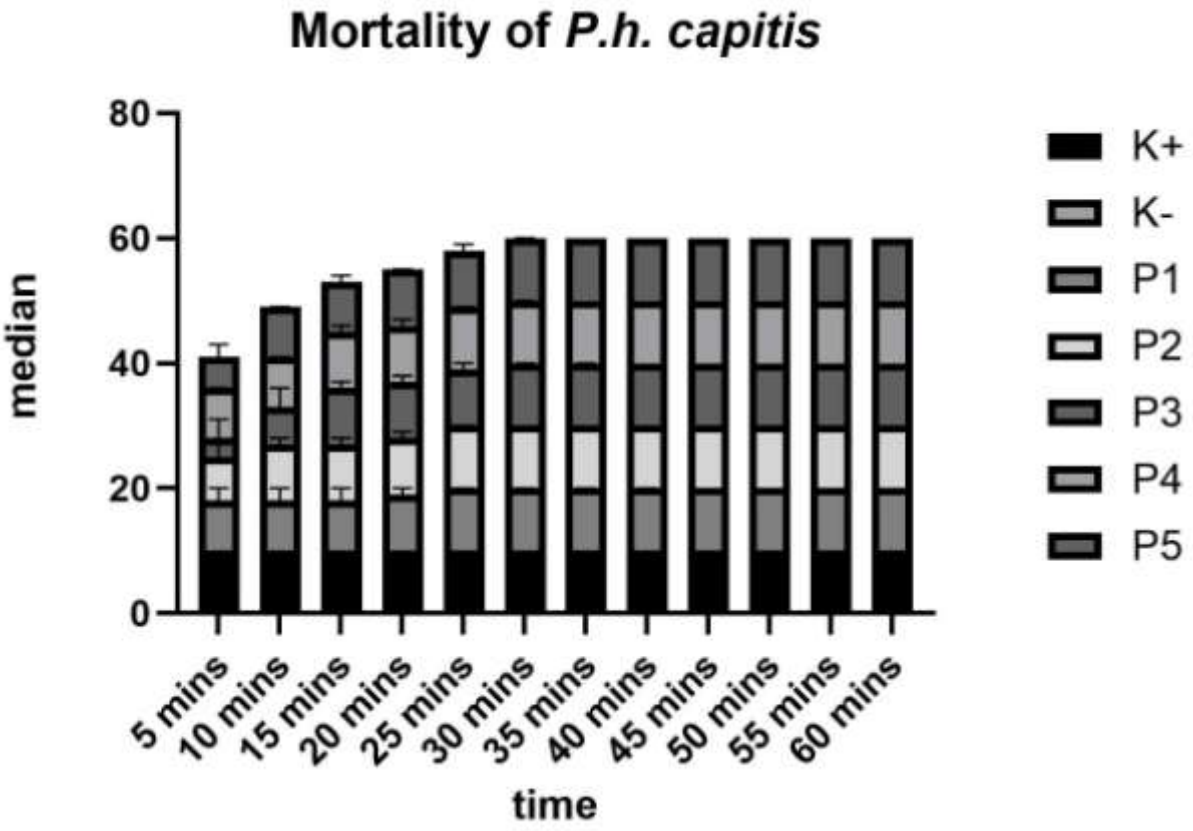

Figure 1. Results of statistical analysis using GraphPad Prism version 10.4.0.

Table 3. The lethal concentration 50 (LC_{50}) values

Time	LC_{50} (%)
5 minutes	4,116
10 minutes	0,836
15 minutes	225,705
20 minutes	19,395
25 minutes	0,001
30 minutes	41,627
35 minutes	3,401
40 minutes	0,847
45 minutes	0
50 minutes	0
55 minutes	0
60 minutes	0

Table 4. The lethal time 50 (LT_{50}) values

Treatment	LT_{50} (minutes)
P1	4,834
P2	2,799
P3	2,462
P4	2,437
P5	2,163

appetite suppressant and fertility disruptor, making it an effective natural insecticide for *P.h. capitis* (Dalming *et al*., 2022; Virgianti and Rahmah, 2016).

The selection of a 5% concentration is based on a scientific approach that considers both the efficacy and safety of natural ingredients as pediculicides. Previous studies by Nurhaini *et al*. (2020) utilized soursop leaf extract at a 25% concentration, while research by Dalming *et al*. (2022) explored varying concentrations of 15%, 20%, 25% and 30%. Although these studies employed higher concentrations, they did not investtigate the potential synergistic effects of combining these natural ingredients. Therefore, this study was aimed to assess whether a combination of soursop leaf extract and eucalyptus oil remains effective as a pediculicide at a lower concentration, thereby minimizing the risk of scalp irritation, particularly for individuals with sensitive skin.

The safety of the combination of soursop leaf extract and eucalyptus oil should be evaluated by determining the LC_{50} and LT_{50} values. The LC_{50} for the combined extract is 0.001% at 25 minutes, indicating that half the population of *P.h. capitis* is killed within this time at this concentration.

The LT_{50} value for the combination in group P5 is 2.163 minutes, demonstrating that it takes only 2.163 minutes to kill half the population of *P.h. capitis* in this group. A decrease in LC_{50} and LT_{50} values corresponds to an increase in toxicity (Kewa *et al*., 2020; Maula and Musfirah, 2022).

CONCLUSION

The study concludes that the combination of soursop leaf extract and eucalyptus oil is highly effective as a natural pediculicide, attributed to the active compounds acetogenin, isoflavone and cineole. The most effective treatment group in eliminating *P.h.* capitis which utilized a 1:2 ratio of 5% soursop leaf extract and 5% eucalyptus oil. The LC_{50} value for this combination against *P.h. capitis* was determined to be 0.001% at 25 minutes, while the LT_{50} value was 2.163 minutes, indicating its rapid and potent pediculicidal activity.

SUGGESTION

Further research is needed using Scanning Electron Microscope to observe the morphological appearance of *P.h. capitis* that has been given a combination of soursop leaf extract and eucalyptus oil as an effective pediculicide. Additionally, a survival analysis should be conducted to determine the most effective natural ingredient in killing *P.h. capitis.*

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