Jurnal Veteriner pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional Peringkat 2, Dirjen Riset dan Pengembangan,
Kemendiktisaintek RI SK No 10/C/C3/DT.05.00/2025, 21 Mar.2025

Desember 2025 Vol. 26 No.4: 492-504
DOI: 10.19087/jveteriner.2025.26.4.492
online pada ttp://ejournal4.unud.ac.id/index.php/juvet

Soursop Leaves Extract Combined with Eucalyptus Oil: A Natural Strategy for Controlling Bed Bugs

(KOMBINASI EKSTRAK DAUN SIRSAK DENGAN MINYAK KAYU PUTIH: SUATU STRATEGI ALAMI DALAM PENGENDALIAN KUTU KASUR)

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ABSTRACT

Insecticides used in controlling Bed bugs (*Cimex lectularius*) often result in side effects and the development of resistance. Betel leaf (*Piper betle* Linn.), soursop leaf (*Annona muricata* Linn.), and Eucalyptus (*Melaleuca cajuputi*) oil have the potential to serve as natural insecticides due to their active ingredient content in the form of flavonoids, tannins, alkaloids and other bioactive compounds. This study aimed to evaluate the potency of ethanol extracts of betel leaf or soursop leaf in combination with cajeput oil in inducing mortality in *C. lectularius*. A true experimental design was employed using a post-test only with control group design, which included a positive control (2% bifenthrin), a negative control (distilled water), and 10 treatment groups with combinations of betel leaf or soursop leaf extracts with Eucalyptus oil in ratios of 1:1, 1:2, 2:1, 1:3, and 3:1. Each group consisted of 10 bed bugs, with three replications (totaling 360 bed bugs). Mortality was observed every five minutes over one hour and analysed using the Friedman test. The results indicated significant differences in the potential of the combinations of ethanol extracts and Eucalyptus oil. Probit analysis was used to determine LC_{50} and LT_{50} values. The LC_{50} for the betel leaf and Eucalyptus oil combination was 1.302%, with an LT_{50} of 0.976 minutes (ratio 1:1). For the soursop leaf and Eucalyptus oil combination, the LC_{50} was 2.229%, and the LT_{50} was 0.792 minutes (ratio 1:2). The fastest mortality was observed at five minutes in the combination of soursop leaf and Eucalyptus oil at a 3:1 ratio.

Keywords: Soursop (*Annona muricata* Linn.); Bed bugs (*Cimex lectularius*); Eucalyptus (*Melaleuca cajuputi*) oil; Betel (*Piper betle* Linn)

ABSTRAK

Insektisida sintetis dalam pengendalian kutu busuk atau *Cimex lectularius* kerap menimbulkan efek samping serta resistansi. Daun sirih, daun sirsak dan minyak kayu putih berpotensi menjadi insektisida alami karena mengandung *flavonoid, tanin, alkaloid*, dan senyawa aktif lainnya. Penelitian ini bertujuan mengevaluasi potensi kombinasi ekstrak etanol daun sirih (*Piper betle* Linn.), ekstrak etanol daun sirsak (*Annona muricata* Linn.), dan minyak kayu putih (*Melaleuca cajuputi*) dalam mematikan *C. lectularius*. Desain penelitian berjenis *true experimental* dengan rancangan *post-test only with control group* meliputi kontrol positif *(bifenthrin* 2%*),* kontrol negatif *(aquadest),*serta 10 kombinasi baik daun sirih dan daun sirsak dengan minyak kayu putih perbandingan 1:1, 1:2, 2:1, 1:3, 3:1. Masing-masing kelompok perlakuan terdiri atas 10 kutu busuk, dengan tiga kali pengulangan (total 360 kutu). Mortalitas diamati setiap lima menit selama satu jam, kemudian dianalisis menggunakan uji Friedman. Terdapat perbedaan potensi kombinasi ekstrak etanol daun sirih atau ekstrak etanol daun sirsak dengan minyak kayu putih. Nilai LC_{50} dan LT_{50} dihitung menggunakan analisis probit. Hasilnya menunjukkan bahwa nilai LC_{50} kombinasi daun sirih dan minyak kayu putih sebesar 1,302% dan LT_{50} 0,976 menit (1:1). Nilai LC_{50} daun sirsak dan minyak kayu putih sebesar 2,229% dan LT_{50} 0,792 menit (1:2). Mortalitas *C. lectularius* tercepat terjadi lima menit pada kombinasi daun sirsak dan minyak kayu putih perbandingan 3:1.

Kata-kata kunci: daun sirsak (*Annona muricata* Linn.); kutu busuk (*Cimex lectularius*.); minyak kayu putih (*Melaleuca cajuputi*), daun sirih (*Piper betle* Linn)

INTRODUCTION

Bed bugs **(***Cimex lectularius*) are ecto-parasites that parasitise humans nocturnally through painless bites, causing a variety of clinical symptoms including pruritus, swelling, erythema, insomnia and psychological distress. Their blood-feeding behaviour can lead to cutaneous manifestations ranging from papular eruptions to severe allergic reactions (Ronawati *et al*., 2022; Akhoundi *et al*., 2023). Additionally, *C. lectularius* is identified as a potential vector for transmitting various pathogens, including viruses, bacteria and parasites associated with diseases such as filariasis, Chagas disease, *Brucella melitensis* infection, yellow fever, Human Immunodeficiency Virus (HIV), hepatitis B and more than 40 other medical conditions (El Hamzaoui *et al*., 2019).

Epidemiological data from 1995 to 2023 indicate that the highest prevalence of *C. lectularius* bites was reported in Uganda (69.9%), followed by Iran (62%), Nigeria (21.8%) and France, where prevalence increased from 7% in 2014 to 11% in 2023. Following the Covid-19 pandemic, a global upward trend in bed bug infections has been observed (Sharififard *et al*., 2020; Gustin, 2024). In Indonesia, the highest prevalence in 2023 was recorded in West Java Province (54.8%), followed by Jakarta (10.8%), Banten (8.3%) and Central Java (3.0%). Notably, Purbalingga Regency in Central Java reported a local prevalence of 9.4% in 2022 (Ronawati *et al*., 2022; Meisyara *et al*., 2023). Additionally, anecdotal accounts from residents in Banyumas and Pemalang indicate the presence of *C. lectularius* in residential areas, although no formal documentation regarding bite incidents has been issued by local authorities.

Control strategies targeting *C. lectularius* frequently involve the incineration of infested materials or the destruction of their nesting sites. As an alternative, chemical treatments are employed to preserve contaminated belongings from physical damage (Bhirich *et al*., 2023). Although synthetic insecticides have demonstrated efficacy in eradicating *C. lectularius*, their

formulations, which often comprise neonicotinoid compounds such as acetamiprid, imidacloprid, or deltamethrin, alongside pyrethroids, piperonyl butoxide, S-methoprene, boric acid, silica (diatomaceous earth), and sulfuryl fluoride, are not without consequence. These chemical agents have been linked to severe health hazards in humans, including cases of toxic agent and mortality, with reported risk levels approaching 99% (Berenji *et al*., 2019; Yu *et al*, 2023). Furthermore, the widespread application of synthetic insecticides has been associated with the development of insecticide resistance and potential genetic mutations in *C. lectularius* populations, with resistance rates documented as high as 93.5% (Sinambela, 2024).

In recent years, there has been a growing interest in the development of natural insecticides as alternative control strategies. Various plant species have been identified to contain bioactive compounds with insecticidal properties, offering a safer and more environmentally friendly alternative to synthetic chemical agents (Setty-Siamtuti *et al*., 2017). Among these, betel leaf (*Piper betle* Linn.) and soursop leaf (*Annona muricata* Linn.) have been widely recognised for their efficacy as botanical insecticides. Both plants are known to possess secondary metabolites such as tannins, alkaloids, polyphenols and flavornoids, which exhibit toxicity against different lice species (Milasari *et al*., 2020; Darlis *et al*., 2024). These compounds exert their insecticidal effects by interfering with the nervous system of the target organisms, particularly by disrupting the transmission of nerve impulses, leading to neuromuscular impairment and eventual death (Gaire *et al*, 2020). Eucalyptus oil, a distillate derived from the Melaleuca plant, has traditionally been utilised in Indonesia for various applications, including as a topical warming agent, insect repellent and respiratory aid. This essential oil contains several active constituents with known insecticidal effects, such as 1,8-cineole (63%), terpinyl acetate (13%), and α-terpineol (11%). The compound 1,8-cineole functions by inhibiting the enzyme acetylcholinesterase wit-hin the lice's nervous system, resulting in continuous neural stimulation, sustained muscle contractions, and ultimately, death due to convulsions (Aripin *et al*., 2022; Irfan *et al*., 2022).

Research exploring the combined insecticidal potential of herbal plant extracts and eucalyptus oil (*Melaleuca* spp.) against *C. lectularius* remains relatively scarce. Several studies have reported the strong effectiveness of essential oils and plant extracts, such as *Tagetes patula*, *Schinus molle*, *Cinnamomum* sp., as well as mixtures of *Capsicum annuum*, *Citrus sinen sis*, *Tagetes minuta*, and *Allium sativum* (Afshar *et al*., 2023; Walukhu and Nyukuri, 2020). However, to date, no studies have specifically evaluated the insecticidal potential of *P. betle* Linn and *A. muricata* Linn against *C. lectularius.* Notably, previous studies have demonstrated that a 15% concentration of betel leaf (*P. betle* Linn.) in shampoo formulations was capable of inducing up to 80% mortality in head lice (*Pediculus humanus capitis*) (Susanti *et al*., 2024). Similarly, the application of soursop leaf (*A. muricata* Linn.) extract at a 5% concentration has been reported to achieve 90% mortality in papaya mealybugs (*Paracoccus marginatus*) (Darlis *et al*., 2024). Additionally, the eucalyptol (1,8-cineole) content in eucalyptus oil exhibits ovicidal and adulticidal activity against *C. lectularius* by damaging the mesothoracic region of the insect's body (Zhang *et al*., 2019). In light of these findings, the present study aimed to evaluate the insecticidal efficacy of betel leaf and soursop leaf extracts in combination with eucalyptus oil as a potential botanical alternative for the control of *C. lectularius*.

RESEARCH METHODS

This study was conducted using a true experimental design, specifically adopting a post-test-only control group design, which included two control groups and ten treatment groups. The control groups

consisted of a positive control group, which received a 2% bifenthrin pyrethroid solution, and a negative control group, which was administered distilled water. The ten treatment groups were divided into two sets based on the type of plant extract used: a combination of 5% ethanol extract of betel leaf (*P. betle* Linn.) with 5% eucalyptus oil (Groups P1–P5), and a combination of 5% ethanol extract of soursop leaf (*A. muricata* Linn.) with 5% eucalyptus oil (Groups P6–P10). Each group received the formulations in varying ratios, as in a previous study by Aripin *et al*., (2022) demonstrad with 1:1, 1:3, and 3:1 ratio formulations. Based on these results, this study preserves these effective ratios while extending the evaluation to additional ratios including: Group P1: Betel leaf *(P. betle* Linn.*)* and eucalyptus oil in a 1:1 ratio; Group P2: Betel leaf *(P. betle* Linn.*)* and eucalyptus oil in a 1:2 ratio; Group P3: Betel leaf *(P. betle* Linn.*)* and eucalyptus oil in a 2:1 ratio; Group P4: Betel leaf *(P. betle* Linn.*)* and eucalyptus oil in a 1:3 ratio; Group P5: Betel leaf *(P. betle* Linn.*)* and eucalyptus oil in a 3:1 ratio; Group P6: Soursop leaf *(A. muricata* Linn.*)* and eucalyptus oil in a 1:1 ratio; Group P7: Soursop leaf *(A. muricata* Linn.*)* and eucalyptus oil in a 1:2 ratio; Group P8: Soursop leaf *(A. muricata* Linn.*)* and eucalyptus oil in a 2:1 ratio; Group P9: Soursop leaf *(A. muricata* Linn.*)* and eucalyptus oil in a 1:3 ratio; Group P10: Soursop leaf *(A. muricata* Linn.*)* and eucalyptus oil in a 3:1 ratio.

Each experimental group consisted of 10 specimens of *C. lectularius*, with three replicates per group, resulting in a total of 360 individual specimens. Mortality observations were conducted at 5-minute intervals over a 60-minute period, following the protocol described by Elbanoby (2019). This study was conducted between May and July 2024 and received prior approval from the Health Research Ethics Committee of Universitas Muhammadiyah Purwokerto, under reference number KEPK/UMP/83/V/2024. Specimens of *C. lectularius* were obtained from infected residential areas located in the Purbalingga and Pemalang districts of Central Java. Preparation of the herbal plant extracts was performed in the Pharmaceutical Biology Laboratory, Faculty of Pharmacy, while the insecticidal bioassays were conducted in the Medical Technology Laboratory, Universitas Muhammadiyah Purwokerto.

Tools and Materials

The equipment utilised in this study comprised tweezers, glass jars, dropper pipettes, measuring cylinders, a blender, a rotary evaporator, a stopwatch, and plastic containers. The materials included betel leaves (*P. betle* Linn.), soursop leaves (*A. muricata* Linn.), eucalyptus oil, 96% ethanol, 2% bifenthrin pyrethroid, distilled water (aquadest), and specimens of *C. lectularius*. Additional materials consisted of tissue paper, white paper, aluminium foil, gauze, sponges and filter paper.

Preparation of Betel Leaf and Soursop Leaf Extract

The drying of betel (*P. betle* Linn.) and soursop (*A. muricata* Linn.) leaves was performed using a drying cabinet until the moisture content decreased and the leaves exhibited a brownish hue. Following the drying process, the leaves were cut into medium-sized pieces and subsequently ground and sieved to obtain a fine powder. A total of 250 g of each leaf powder was weighed. The powder was then subjected to maceration by placing it into a maceration vessel, followed by the addition of 96% ethanol at a ratio of 1 g of powder to 10 mL of ethanol, yielding a total volume of 2,500 mL of ethanol (Noer *et al.*, 2018).

Maceration was conducted by placing the plant material in a closed container and storing it away from direct sunlight. The mixture was allowed to stand for a minimum of three days, with occasional stirring to facilitate extraction. Upon completion of the maceration process, the mixture was filtered using filter paper to obtain a 5% ethanol extract of betel (*P. betle* Linn.) leaf. The filtrate was then subjected to rotary evaporation to remove the 96% ethanol, resulting in a concentrated and

purer extract. The same extraction procedure was applied to soursop (*A. muricata* Linn.) leaf (Buulolo, 2023).

Determination of Herbal Extract Concentrations

Each 5% solution of betel leaf extract, soursop leaf extract and eucalyptus oil was prepared by measuring 5 mL of the respective pure substance into separate containers, followed by the addition of distilled water (aquadest) to a final volume of 100 mL. The 5% betel leaf extract was then combined with 5% eucalyptus oil at five different ratios: 1:1 (5 mL : 5 mL), 1:2 (5 mL : 10 mL), 2:1 (10 mL : 5 mL), 1:3 (5 mL : 15 mL), and 3:1 (15 mL : 5 mL). An identical mixing procedure was employed for the combination of 5% soursop leaf extract and 5% eucalyptus oil (Susanti *et al*., 2024).

Collection of *C. lectularius* Specimens

Adult specimens of *C. lectularius* were collected from beds, sofas and other damp areas in several residents' homes. These insects typically range from 1–5 mm in length and 1–3 mm in width, with a dorsoventrally flattened, oval-shaped body covered in fine hairs (Saady, 2023). The female reproductive structure, known as the spermalege, is bilaterally paired and located on the left side of the fifth abdominal segment between the lateral and central regions. In contrast, the male reproductive organ is situated between the eighth and ninth segments (Kawasima *et al*., 2022). Following collection, the bed bugs were transferred from the collection site to the testing location using a closed, humid container protected from direct sunlight. To minimise stress and maintain viability, the transfer was preferably carried out at night. Once in the test location, bed bugs were housed in jars containing circular sponges and gauze to simulate natural hiding environments. The jars were maintained in a dark environment at ambient room temperature (25 ± 2°C) and relative humidity of 40 ± 15%. The insects were fed daily with human blood and plasma for four consecutive days. Under these controlled conditions, *C. lectularius* can survive for several weeks and are capable of reproducing within 5–6 days (Elbanoby, 2019; Zhang *et al*., 2019; Yu *et al.*, 2023).

Testing of Control and Treatment Groups

Twelve plastic containers were prepared and labelled as follows: positive control, negative control, and ten treatment groups (P1 through P10). Each container was lined with filter paper. The positive control received 10 mL of a 2% bifenthrin pyrethroid solution, while the negative control was treated with 10 mL of distilled water (Aquadest). Containers P1 through P5 were treated with various ratios of a 5% ethanol extract of betel leaf (*P. betle* Linn.) combined with eucalyptus oil, whereas containers P6 through P10 were treated with corresponding combinations of a 5% ethanol extract of soursop leaf (*A. muricata* Linn.) and eucalyptus oil (Buulolo, 2023). All treatment solutions were thoroughly homogenised to ensure even distribution within the container. Subsequently, 10 adult *C lectularius* individuals were introduced into each container. The activity and mortality of the insects were monitored at 5-minute intervals over a 60-minute observation period. To ensure reliability and reproducibility, the experimental procedure was replicated three times (Gaire *et al.*, 2020).

Calculation of *C. lectularius* Mortality

At this stage, the mortality of *C. lectularius* was assessed by recording the number of dead specimens at each predetermined time interval, specifically every five minutes for a duration of one hour (Gaire *et al.*, 2020). Each dead insect was considered a single mortality event, contributing to the overall mortality rate calculation. The percentage of mortality was determined using the following formula:

$$\text{Mortality (\%)} = (\text{Number of Dead Bugs}) \times (\text{Total Number of Bugs})^{-1} \times 100\%.$$

This calculation was applied to each treatment and control group across all observation time points. To ensure the

reliability and consistency of the data, the observation procedure was repeated for all groups (Aripin *et al.*, 2022).

Calculation of LC_{50} and LT_{50} Values of *C. lectularius*

The mortality of *C. lectularius* was recorded at 5-minute intervals over a 60-minute observation period. The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were determined through probit analysis, based on the percentage of mortality observed across treatment concentrations (Kresnadi and Rachmawati, 2021).

Data Analysis

Differences in effectiveness between observation times were evaluated using the Repeated Measure ANOVA test and the Post Hoc Tukey test with GraphPad Prism 10.4.0. The LC50 and LT50 values were analysed using probit analysis.

RESULTS AND DISCUSSION

Based on the data presented in Table 1, the treatment groups 5% betel leaf extract and 5% eucalyptus oil (1:1) (P1), 5% betel leaf extract and 5% eucalyptus oil (1:2) (P2), % betel leaf extract and 5% eucalyptus oil (1:3) (P4), and 5% betel leaf extract and 5% eucalyptus oil (3:1) (P5), namely the treatment of betel leaf extract and eucalyptus oil, demonstrated a high insecticidal potential, each achieving 100% mortality of *C. lectularius* within the first 15 minutes of exposure.

As shown in Table 2, the combination treatment of soursop leaf extract and eucalyptus oil in group P10 exhibited strong insecticidal activity, resulting in 100% mortality of *C. lectularius* within the first five minutes of observation.

Figure 1 presents, the analysis results, revealing that the treatment group had a highly significant impact on insect mortality ($F = 3842.32$; $p<0.0001$), explaining 98.63% of the total variance. This indicates that the treatment type is the primary determinant of mortality outcomes. Although exposure duration also had a statistically significant effect ($F = 7,14$; $p<0.0001$), its contribution to the overall variance was minimal (0,336%). The interaction between treatment and exposure duration was no statistically significant ($F = 1,11$; $p < 0.2927$), accounting for 0.3137% of the total variance. These findings suggest that the effect of the treatment group on insect mortality was consistent throughout the observation period and not influenced by the exposure duration.

Figure 2 presents, the analysis results, indicating that the treatment group had a highly significant impact on insect mortality ($F = 6713.12$; $p < 0.0001$), accounting for 98.89% of the total variance. This highlights the dominant role of treatment type in influencing insect mortality. The exposure duration factor also demonstrated a statistically significant effect ($F = 8.78$; $p < 0.0001$); however, its contribution to the total variance was relatively minimal, at only 0.2372%. Additionally, the interaction between treatment and exposure duration was found to be statistically significant ($F = 2.85$; $p < 0.0001$), contributing 0.461% to the overall variance. These results suggest a meaningful interaction between the two variables, indicating that the effectiveness of a treatment is influenced by the duration of exposure and vice versa. Consequently, interpreting the main effects of each factor in isolation may lead to misleading conclusions.

According to the LC_{50} values presented in Table 3, the combination of 5% betel leaf extract and 5% eucalyptus oil at a concentration of 1.302% demonstrated the highest efficacy, effectively causing 50% mortality of *C. lectularius* within 5 minutes. This suggests that this formulation has significant potential as a rapid-acting insecticidal agent. Similarly, the LC_{50} value for the combination of 5% soursop leaf extract and 5% eucalyptus oil was determined to be 2.229%, indicating that this mixture also possesses considerable potential to achieve 50% mortality of *C. lectularius* within the

Table 1. Mean percentage of *Cimex lectularius* mortality from betel leaf and eucalyptus oil

Minutes	Treatment						
	K+	K-	P1	P2	P3	P4	P5
5	100	0	87	87	90	97	93
10	100	0	97	87	93	97	97
15	100	0	100	100	93	100	100
20	100	0	100	100	100	100	100
25	100	0	100	100	100	100	100
30	100	0	100	100	100	100	100
35	100	0	100	100	100	100	100
40	100	0	100	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+ (2% bifenthrin pyrethroid), K– (aquadest), P1: 5% betel leaf extract and 5% eucalyptus oil (1:1), P2: 5% betel leaf extract and 5% eucalyptus oil (1:2), P3: 5% betel leaf extract and 5% eucalyptus oil (2:1), P4: 5% betel leaf extract and 5% eucalyptus oil (1:3), P5: 5% betel leaf extract and 5% eucalyptus oil (3:1).

Table 2. Mean Percentage of *C. lectularius* Mortality from Soursop leaf and Eucalyptus oil

Minutes	Treatment						
	K+	K-	P6	P7	P8	P9	P10
5	100	0	77	97	93	93	100
10	100	0	87	100	97	97	100
15	100	0	100	100	100	100	100
20	100	0	100	100	100	100	100
25	100	0	100	100	100	100	100
30	100	0	100	100	100	100	100
35	100	0	100	100	100	100	100
40	100	0	100	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+ (2% bifenthrin pyrethroid), K– (aquadest), P1: 5% soursop leaf extract and 5% eucalyptus oil (1:1), P2: 5% soursop leaf extract and 5% eucalyptus oil (1:2), P3: 5% soursop leaf extract and 5% eucalyptus oil

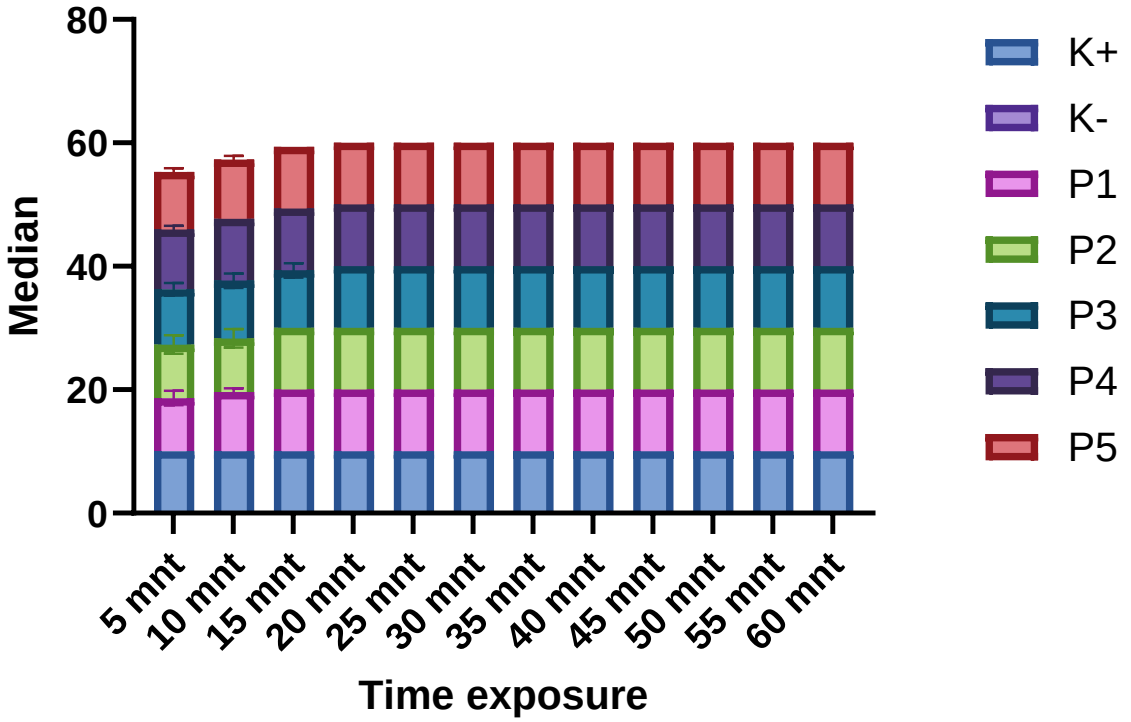

Figure 1. Diagram Statistical Analytic of *C. lectularius* Mortality from Betel leaf and Eucalyptus oil

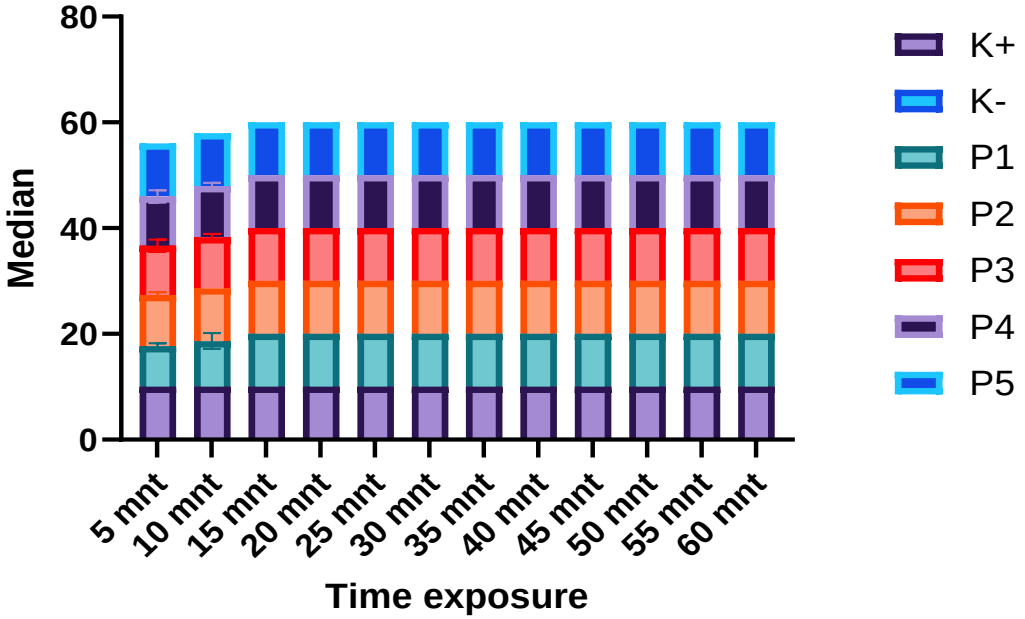

Figure 2. Diagram Statistical Analytic of *C. lectularius* Mortality from Soursop leaf and Eucalyptus oil

same observation period. Based on the LT_{50} values presented in Table 4, the combination of 5% betel leaf extract and 5% eucalyptus oil at a 1:1 ratio exhibited the highest time-based efficacy, with an LT_{50} of 0.976 minutes.

This result indicates that the formulation is capable of inducing 50% mortality in *C. lectularius* within approximately one minute. Similarly, the combination of 5% soursop leaf extract and 5% eucalyptus oil at a 1:2 ratio demonstrated an LT_{50} value of 0.792 minutes, signifying a slightly faster lethal effect under the same conditions. These findings highlight the rapid insecticidal potential of both extract combinations against *C. lectularius*.

This study revealed that all tested treatments achieved complete (100%) mortality of *C. lectularius*. The most rapid mortality was observed in treatment P10, consisting of a combination of 5% soursop leaf extract and 5% eucalyptus oil, which induced total mortality in less than five minutes. In contrast, the combination of 5% betel leaf extract and 5% eucalyptus oil produced 100% mortality within less than 20 minutes. These results are consistent with findings by Susanti *et al*. (2024), who reported that a shampoo containing 30% betel leaf extract achieved 100% mortality of head lice (*P.h. capitis*) within five minutes. Similarly, Ninuk *et al*. (2024) demonstrated that soursop leaf extract elicited complete mortality of head lice (*P. h. capitis*) within 15 minutes. Additionally, studies by Afshar *et al*., (2023) on *C. lectularius* have also reported high mortality rates, with *Tagetes patula, Schinus molle*, and *Cinnamomum* sp. causing up to 100% mortality, while *Ricinus communis* extract induced 92.31% mortality (Saady, 2023).

The observed insecticidal effects in this study are likely attributed to the synergistic action of secondary metabolites present in betel and soursop leaf extracts, including flavonoids, tannins, alkaloids, polyphenols, and saponins. These compounds have been reported to disrupt cellular integrity, inhibit enzymatic activity and interfere with nerve impulse transmission, leading to neuromuscular dysfunction and eventual mortality in *C. lectularius* (Milasari *et al*., 2020; Wahyuni and Loren, 2015; Kresnadi and Rachmawati, 2021; Shalsadila *et al*., 2023; Utami and Porsia, 2023).

Tabel 3. LC_{50} Value of *C. lectularius* Mortality Activity from the Combination of Herbal Plants and Eucalyptus oil

Time	LC_{50} (%)	
	EDS + MKP	EDR + MKP
5 Minutes	1,302	2,229
10 Minutes	2,471	4,270
15 Minutes	0,875	3,178
20 Minutes	1,641	2,771
25 Minutes	1,625	2,680
30 Minutes	1,625	2,680
35 Minutes	0	0
40 Minutes	0	0
45 Minutes	0	0
50 Minutes	0	0
55 Minutes	0	0
60 Minutes	0	0

Description: LC (*Letal Consentration*), EDS (Betel Leaf Extract), EDR (Soursop Leaf Extract) MKP (Eucalyptus oil).

Additionally, the active constituents found in eucalyptus oil, such as 1,8-cineole, terpinyl acetate, and α-terpineol, have been demonstrated to inhibit acetylcholinesterase activity in the insect nervous system, compromise membrane stability, suppress enzymatic function and interfere with genetic material (Joen, 2020; Aripin *et al*., 2022; Irfan *et al*., 2022). This is consistent with the findings of Saady (2023), who reported that eucalyptus oil was capable of inducing a mortality rate of 78.32% in *C. lectularius*.

This study demonstrated that the LC_{50} value for *C. lectularius* mortality was 1.302% following treatment with a combination of 5% ethanol extract of betel leaf

and 5% eucalyptus oil. In contrast, the LC_{50} value was 2.229% after treatment with a combination of 5% ethanol extract of soursop leaf and 5% eucalyptus oil. These findings align with the study by Gaire *et al.* (2019), which reported that the eucalyptol content in herbal plants exhibited an LC_{50}

Tabel 4. LT_{50} Value of *C. lectularius* mortality Activity from the Combination of Herbal Plants and Eucalyptus oil

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Time		
	EDS + MKP	EDR + MKP
P1	0,976	2,015
P2	2,435	0,792
P3	3,211	1,444
P4	2,438	1,444
P5	1,444	0

Description : LT (Lethal Time), EDS (Betel Leaf Extract), EDR (Soursop Leaf Extract), MKP (Eucalyptus oil). P1 (ratio of 5% herbal extract and 5% eucalyptus oil at 1:1), P2 (ratio of 5% herbal extract and 5% eucalyptus oil at 1:2), P3 (ratio of 5% herbal extract and 5% eucalyptus oil at 2:1), P4 (ratio of 5% herbal extract and 5% eucalyptus oil at 1:3), P5 (ratio of 5% herbal extract and 5% eucalyptus oil at 3:1).

value of 191.1% against *C. lectularius*. The LT_{50} values indicated that the combination of 5% ethanol extract of betel leaf and 5% eucalyptus oil at a 1:1 ratio induced mortality in *C. lectularius* within 0.976 minutes. In comparison, the combination of 5% ethanol extract of soursop leaf and 5% eucalyptus oil at a 1:2 ratio produced an LT_{50} value of 0.792 minutes against *C. lectularius*. Consistently, a study by Kresnadi and Rachmawati (2021) reported that the active compounds in betel leaf exhibited an LT_{50} value causing 50% mortality of the brown stink bug (*Riptortus linearis*) within 26.8 hours.

The efficacy of the combination of 5% ethanol extract of soursop leaf and 5% eucalyptus oil may be attributed to several factors. Soursop leaf contains acetogenin compounds, which are not found in betel leaf; these compounds exert toxicity through direct contact with the insect's body, ultimately inhibiting ATP synthesis and causing cell death (Pradana *et al.*, 2023). Moreover, the treatment group P10, with a 3:1 ratio, exhibited synergistic interactions among its compounds, accelerating mortality in *C. lectularius*. However, as highlighted by Gaire *et al.* (2020), the potency of such combinations may decrease if antagonistic effects occur, particularly when the formulation is not accurate. Additionally, eucalyptus oil primarily inhibits acetylcholinesterase enzymes and does not directly toxicize the insect; therefore, if the eugenol and chavicol content in betel leaf is weakened by 1,8-cineole, the overall insecticidal efficacy may be reduced (Tiensi and Sulaiman, 2018).

CONCLUSION

The combination of ethanol extract of betel leaf and eucalyptus oil, as well as the combination of ethanol extract of soursop leaf and eucalyptus oil, shows promising potential for controlling *C. lectularius*. The fastest mortality was observed with the combination of ethanol extract of soursop leaf and eucalyptus oil at a 3:1 ratio, achieving complete mortality in less than 5 minutes.

SUGGESTION

To optimize the efficacy and safety of the combination of herbal extracts and eucalyptus oilagainst *C. lectularius* within a short exposure time, higher concentrations and more suitable application formulations, such as sprays or aerosols, should be evaluated. Additionally, field testing should be conducted to assess the potential effectiveness of these treatments under real-world conditions in household or bedding environments.

ACKNOWLEDGEMENT

Thanks to the Research and Community Service Division of the University of Muhammadiyah Purwokerto for funding this research through the Fundamental II Grant Scheme, No. A.11-III/7473-S.Pj./LPPM/II/

2024.

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