Research Article

In Vitro and *In Vivo* Acaricidal Activity of *Senna alata* Leaves Extract Against Rabbit Ear Mites, *Psoroptes cuniculi*

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Abstract | In this study, the effectiveness of *Senna alata* leaf extract against rabbit ear mites, *Psoroptes cuniculi*, was evaluated using both *in vitro* and *in vivo* methods. The results demonstrated a clear correlation between the extract concentration and mite mortality rate. Higher concentrations of the extract *in vitro* resulted in increased mite mortality, with specific concentrations causing 50% (LC_{50}) and 90% (LC_{90}) mortality identified as 282.45 mg/ml and 478.71 mg/ml, respectively. *In vivo* tests showed significant reductions in mite infestation with extract concentrations of 160 mg/ml and 200 mg/ml. The 200 mg/ml concentration exhibited slightly higher efficacy compared to 160 mg/ml. The mean infestation level decrease for concentration of 200 mg/ml from 2.5 ± 0.4 on Day 0 to 1.05 ± 0.23 by Day 7. These findings indicate that *S. alata* leaf extract has strong acaricidal properties and could potentially serve as a natural and sustainable alternative to synthetic acaricides for managing rabbit ear mite infestations.

Keywords | Biopesticide, Ear mange, Gelenggang, Phytochemicals, Sustainable, Terengganu

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INTRODUCTION

Animal ectoparasites are parasites that reside on the exterior of their animal hosts, presenting significant challenges to animal welfare across various hosts such as livestock, pets, and wildlife. Mites, eight-legged arthropods belonging to the class Arachnida, are among the notable ectoparasites affecting animals (Bowman, 2009). Mites of the genus *Psoroptes* are particularly notorious for causing severe dermatological issues. *Psoroptes cuniculi*, a prevalent ectoparasite found in rabbits, is responsible for causing ear mange (Ulutas *et al*., 2005) (Figure 1). This condition is characterized by intense itching, inflammation, and secondary bacterial infections. Infestation by *P. cuniculi* causes physical discomfort and poses health risks to affected rabbits. A study by Mohamad-Radzi *et al*. (2021) reported a prevalence of mite infestations exceeding 50% in rabbit farming in Selangor, Malaysia. Such infestations lead to economic losses in commercial rabbit farming by reducing production and increasing veterinary expenses.

Traditionally, chemical acaricides have been employed to control *Psoroptes cuniculi* infestations. However, excessive and improper use of synthetic acaricides has led to the development of resistance in mite populations, environmental pollution, and residues of chemicals in animal products (Seddiek *et al*., 2013). These challenges underscore the need to explore alternative control methods that are effective and environmentally sustainable. Plant-derived compounds have garnered attention as promising alternatives due to their bioactive properties and minimal environmental impact (Nwanade *et al*., 2022).

Figure 1: *Psoroptes cuniculi* (Delafond, 1859).

Senna alata, also known as *Cassia alata*, is a flowering shrub belonging to the Fabaceae family. Native to South America, it is commonly found in tropical regions, including Malaysia (Gritsanapan and Mangmeesri, 2009). *Senna alata* is known by various local names such as candle bush, ringworm bush, 'ketepeng cina', and in Malaysia, it is referred to as 'gelenggang'. This shrub typically grows to a height of 1-2 meters, occasionally reaching up to 5 meters. Gelenggang is distinguished by its vibrant yellow inflorescence resembling candlesticks, which gives rise to its common name (Figure 2).

Figure 2: *Senna alata* (L.) Roxb. Plant.

Senna alata, known for its medicinal properties, has been traditionally used to treat various ailments including typhoid, diabetes, malaria, asthma, ringworms, tinea infections, scabies, blotch, herpes, and eczema (Fatmawati, 2020). This plant exhibits a wide array of biological activities attributed to its components such as flowers, roots, leaves, stems, seeds, and bark. These activities include antioxidant, anti-inflammatory, abortifacient, antimicrobial, antiallergic, antilipogenic, antibacterial, antidiabetic, antifungal, anticancer, and antiviral properties (Oladeji *et al*., 2020). Locally in Malaysia, boiled Gelenggang leaves have been traditionally used to treat rabbits with ear mite infections.

The bioactive properties of *S. alata* are attributed to phytochemical constituents extracted from the plant, including flavonoids, phenolics, alkaloids, anthraquinones, tannins, terpenoids, and steroids (Angelina *et al*., 2021). A previous study has assessed the acaricidal efficacy of *S. alata* leaf extract against *Rhipicephalus annulatus*, a common tick species in cattle (Ravindran *et al*., 2012), and it was suggested that anthraquinone may contribute to the acaricidal activity of *S. alata* extracts due to its anti-mite properties (Phongpaichit *et al*., 2004).

Despite its extensive traditional medicinal use, the potential acaricidal effects of *S. alata* against *P. cuniculi*, particularly in rabbits, have not been extensively studied. An *in-silico* study has been conducted using phytochemicals from *S. alata* against two targeted proteins of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*, suggesting potential herbal acaricidal properties (Priyadarshini *et al*., 2019). However, further *in vitro* and *in vivo* studies are needed to validate these findings. Therefore, this study aims to evaluate the acaricidal activity of *Senna alata* leave extract against rabbit ear mites, *Psoroptes cuniculi*, using both *in vitro* and *in vivo* approaches.

MATERIALS AND METHODS

Ethical Approval

This study adhered to ethical guidelines approved by the UniSZA Animal and Plant Research Ethics Committee (UAPREC). The research strictly followed UAPREC's protocols (UAPREC/008/020) to ensure minimal distress to the animals involved in the study.

Study Area

This study was conducted at the Hatchery and Laboratory facilities at the Universiti Sultan Zainal Abidin (UniSZA), Besut Campus, Terengganu, Malaysia.

Preparation of *Senna Alata* Leaves Extract

Senna alata leaves were harvested in Besut Terengganu (5°45'20.7"N 102°35'30.9"E) from March 2024 to May 2024. Old leaves were collected for this study. Maturity of the leaves was classified following a method by Dian Nashiela *et al*. (2016). After harvesting, the leaves were washed and air-dried for a week at 40°C, following the method described by Gritsnapan and Mangmeesri (2009). Subsequently, the dried leaves were finely ground into a powder. About twenty dry leaves of *S. alata* (n=20) were used to produce 10 g of powder form.

To prepare the extract, 10 g of powdered *S. alata* leaves were macerated in 100 ml of distilled water. The maceration process was conducted at 55°C for 120 minutes, as detailed by Karthika *et al.* (2016). The extraction procedure was repeated until complete exhaustion of the plant material. The combined maceration extracts were then filtered and dried to completeness using a rotary evaporator.

Mites Collection

New Zealand white rabbits weighing approximately 3-4 kg, afflicted with *Psoroptes cuniculi* infections, were sourced from Expert Rabbitry Global Empire in Kampung Tempinis, Besut, Terengganu. These rabbits were subsequently housed in designated hatchery facilities at Besut Campus, UniSZA.

Mites were collected using a skin scraping method adapted from Abdisa (2018) with slight modifications. The affected skin areas were vigorously scraped six to seven times to remove the top layer of the papules. The collected scabs containing mites were transferred into petri dishes and examined under a stereo microscope to isolate the mites.

In vitro Acaricidal Activity

Mite bioassays were conducted in triplicate following the method described by Fichi *et al.* (2007). The assay included seven treatments:

- Positive control (Doramectin + distilled water).
- T0 (distilled water only as negative control).
- T1 (40 mg/ml of *Senna alata* extract in distilled water).
- T2 (80 mg/ml of *S. alata* extract in distilled water).
- T3 (120 mg/ml of *S. alata* extract in distilled water).
- T4 (160 mg/ml of *S. alata* extract in distilled water).
- T5 (200 mg/ml of *S. alata* extract in distilled water).

Each solution was evenly spread over the surface of a 10 cm diameter petri dish using a sterile swab. Twenty adults of *Psoroptes cuniculi* mites (n=20) were then placed in each petri dish. The plates were kept at room temperature, and mite mortality was recorded every two hours for 24 hours. Mites were considered dead if they showed no reaction when stimulated with a needle.

The efficacy of *S. alata* extract against *Psoroptes cuniculi* was evaluated by calculating the percentage of mite mortality following the methodology outlined by Ravindran *et al.* (2012).

In vivo Acaricidal Activity

For *in vivo* study, only two concentrations of *Senna alata* extract were prepared based on the *in vitro* study results. *In vivo* test were conducted in four treatments: 160 mg/ml (T1) and 200 mg/ml (T2) of *S. alata* extract. For comparison, a negative control group that received only distilled water (NC) and a positive control group that was treated with 10 mg/ml doramectin injections (PC) were also included (Seddiek *et al.*, 2013).

To evaluate the acaricidal activity through *in vivo* testing, 20 domestic rabbits (n=20) that were naturally infected with *Psoroptes cuniculi* were selected. These rabbits were then randomly divided into four groups of five, ensuring a balanced representation. Each group received 2 ml of the treatment solution, which was applied topically to the both left and right of the infected ears of the rabbits (Yipel *et al.*, 2016).

The degree of infestation was evaluated for seven days on the basis of the following scoring system by Guillot and Wright (1981) as shown in Table 1 and Figure 3.

Table 1: Degree of infestation scoring system.

Score	Infestation Stage
0	Absence of scabs and/or mites
0.5	Irritation in the ear canal but no mites observed
1	Small number of scabs in the ear canal, mites present
2	External ear canals filled with scabs, mites present
3	Scabs in the ear canal and proximal 1/4of the pinna, mites present
4	1/2pinna filled with scabs, mites present
5	3/4of the pinna filled with scabs, mites present
6	All internal surfaces of the pinna are full of scabs, and mites present

Table presents the list of degree of infestation for the scoring system of mite's infestation on rabbit's ears.

Figure 3: Degree of infestation on rabbit's ear.

Statistical Analysis

Regression analysis was conducted in Microsoft Excel. Probit analysis and one-way analysis of variance (ANOVA) were conducted using Minitab software. The results are expressed as means ± standard error (SE). Group comparisons among the extract were performed using the Tukey test, with significances at $P < 0.05$. Graphs were created using Origin software.

RESULTS AND DISCUSSION

Acaricidal Activity of *Senna Alata* Leaves Extract *In Vitro*

Figure 4 illustrates the outcomes of the *in vitro* tests using *Senna alata* leaf extract. The effectiveness of the extract against *Psoroptes cuniculi* was evaluated by determining the percentage of mite mortality. The extract's acaricidal activity was tested across various concentrations ranging from 40 mg/ml to 200 mg/ml.

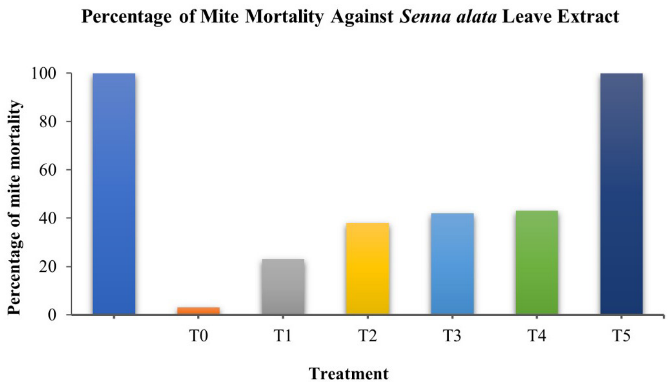

Figure 4: Percentage of mite mortality against *Senna alata* leave extract.

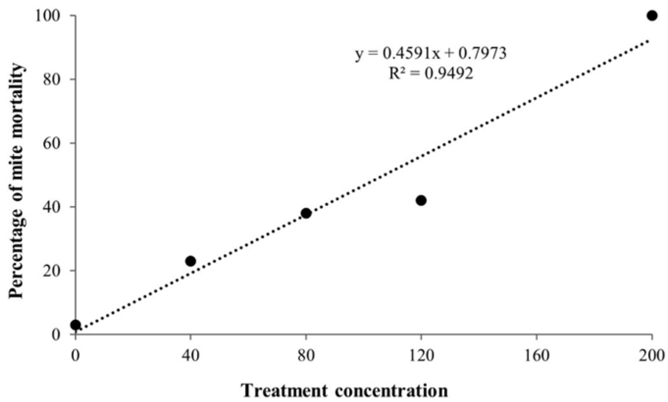

Figure 5: The relationship between the percentage of mite mortality and the concentration of *Senna alata* leave extract.

At the lowest concentration tested, 40 mg/ml, the mortality rate was 23%, which notably increased to 38% at 80 mg/ml. Further increments in concentration to 120 mg/ml and 160 mg/ml results mortality rates of 42% and 43%, respectively, suggesting a potential plateau effect. The highest concentration tested, 200 mg/ml, achieved a maximum mortality rate of 100%, confirming that higher concentrations are more effective in increasing mite mortality.

Results from the regression analysis demonstrated a significant positive correlation between the concentration of treatment and the mortality rate of *Psoroptes cuniculi* with the R^2 value of 0.9492 as shown in Figure 5. This means that 94.92% of the variation in mite mortality is due to the treatment concentration. The findings show that as the dosage of the extract increases, so does the mortality rate of the mites, demonstrating the treatment's efficacy in a dose dependent manner.

Figure 6 illustrates the relationship between the dosage of *S. alata* extract and mite mortality. The graph demonstrates that as the dosage of the extract increases, so does the mortality rate of the mites. The probit regression curve exhibits a steep slope significantly increasing in mite mortality at higher concentrations. The botanical extract from *S. alata* displays substantial acaricidal activity, with specific lethal concentrations for 50% and 90% mortality (LC_{50} and LC_{90}) calculated at 282.45 mg/ml and 478.71 mg/ml, respectively.

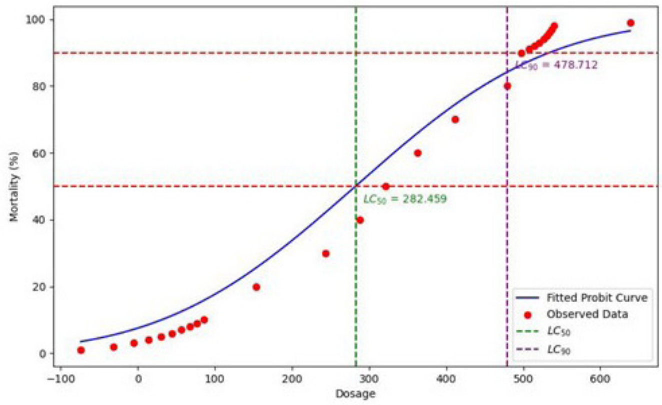

Figure 6: Probit regression (Dose-Response analysis).

Acaricidal Activity of *Senna Alata* Leaves Extract *In Vivo*

Figure 7 illustrates the degree of infestation observed across all four treatments over a seven-day period. In Figure 7a, the positive control group treated with doramectin, displaying a substantial decrease in mite infestation throughout the treatment period. The infestation level decreased progressively, indicating the efficacy of the standard treatment. By the end of the seven days, the infestation was nearly eradicated, validating doramectin as an effective benchmark for assessing the efficacy of *Senna alata* extracts.

The *in vitro* testing of *Senna alata* leaf extract in this study demonstrates its acaricidal properties against *Psoroptes cuniculi*. These findings are consistent with previous research, such as Ravindran *et al*. (2012), which showed the acaricidal activity of *S. alata* leaf extract against cattle ticks. The study reveals a dose-dependent acaricidal effect of *S. alata* extract, where higher concentrations lead to quicker mite mortality. Similar results were observed in a study by Magano *et al*. (2008) using *Senna italica* root extract, where increased extract concentrations correlated with higher mortality rates of the Mediterranean *Hyalomma* tick, *Hyalomma marginatum rufipes*.

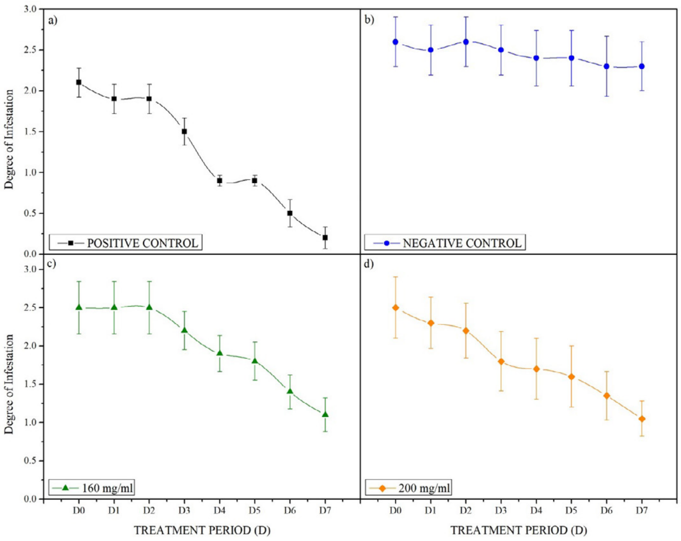

Figure 7: Effect of different treatment group against the degree of infestation of rabbit ear mites over the treatment period of 7 days.

Table 2: Degree of infestations score of *Psoroptes cuniculi* for different concentrations of *Senna alata* leaves extract

Concentration (mg/ml)	Mean Degree of Infestation ± SE							
	D0	D1	D2	D3	D4	D5	D6	D7
Negative Control (Distilled water)	2.6± 0.31^{a}	2.6± 0.31^{a}	2.6± 0.31^{a}	2.5 ± 0.25^{a}	2.4 ± 0.23^{a}	2.4 ± 0.25^{a}	2.3 ± 0.22^{a}	2.3 ± 0.22^{a}
160	2.5± 0.34^{a}	2.5± 0.34^{a}	2.5± 0.34^{a}	2.2 ± 0.25a	1.9 ± 0.23^{ab}	1.8 ± 0.25^{ab}	1.4 ± 0.22^{ab}	1.1 ± 0.22^{b}
200	2. ± 0.4^{a}	2.3± 0.33^{a}	2.2 ± 0.36^{a}	1.8 ± 0.39^{a}	1.7 ± 0.4^{ab}	1.6 ± 0.4^{ab}	1.35± 0.32^{ab}	1.05± 0.23^{bc}
Positive Control (Doramectin)	2.1 ± 0.18^{a}	1.9 ± 0.18^{a}	1.9 ± 0.18^{a}	1.5 ± 0.17^{a}	0.9 ± 0.07^{b}	0.9 ± 0.07^{b}	0.5 ± 0.17^{b}	0.2 ± 0.13^{c}

[1]/ Values are Mean ± SE, n = 10 in each group values in a column with different superscripts differ, (P>0.05). Table presents the results of degree of infestation of the ear mites at different concentration of Senna alata extract.

The probit regression analysis provides critical insight into the efficacy of *S. alata* as botanical pesticides, with the lethal concentration for 50% value (LC_{50}) calculated at 282.45 mg/ml. However, the LC_{50} value of *S. alata* leave extracted in distilled water in this study was three times higher than the previous study by Sunil *et al*. (2013). In the study, the LC_{50} value calculated for *Cassia fistula* leaves extracted in ethanol to kill a tick species, *Rhipicephlaus* (*Boophilus*) *annulatus* was only 97.1 mg/ml. Halim-Lim *et al*. (2020) illustrate that the total phenolic content in *S. alata* leaves was greater in ethanolic extraction than in water extraction. This is because the solvents and extraction techniques utilized in plant extraction are critical factors that substantially affect the yield of bioactive compounds (Abubakar and Haque, 2020). For this reason, further research on various solvents for extraction is necessary to enhance the efficacy of *S. alata* as botanical insecticides, thus optimize its toxic effects on pests at lower lethal concentrations.

Evaluation of acaricidal activity through *in vivo* studies demonstrated significant reductions in the degree of infestation for both concentrations of *S. alata* leaf extract. The progression of mite infestation over seven days highlighted the temporal dynamics of the extract's effectiveness. The slight superiority of the 200 mg/ml concentration over the 160 mg/ml concentration is statistically significant, highlighting its potential as the optimal concentration for practical use as presented in Table 2. These findings align with studies involving *Senna italica* root extract, emphasizing that higher concentrations of botanical extracts enhance their efficacy against mite infestations due to increased potency of phytochemicals (Ravindran *et al*., 2012). The infestation level in the 200 mg/ml group decreased from 2.5 ± 0.4 on Day 0 to 1.05 ± 0.23 by Day 7, indicating prolonged efficacy of *S. alata* extract over time.

The notable decrease in mite infestation during the treatment period indicates that *S. alata* extract may function as a sustainable and efficacious acaricidal agent. The maintained efficacy over seven days suggests the potential for extended protection, decreasing the necessity for frequent reapplications and alleviating stress on treated animals. The temporal efficacy patterns indicate that strategically scheduling applications may enhance outcomes, especially when synchronized with the critical stages of the mite life cycle. These findings emphasize the necessity of evaluating both the immediate impacts and the long-term impact of treatments to comprehensively understand its potential.

Both *in vitro* and *in vivo* results indicate that crude extracts of *Senna alata* leaves possess potent acaricidal properties, attributed to their diverse phytochemical constituents. Previous studies have identified various bioactive components in *S. alata*, including flavonoids, phenolics, alkaloids, anthraquinones, tannins, terpenoids, and steroids, contributing to a range of pharmacological activities (Angelina *et al*., 2021). Characterization of these constituents is crucial for understanding the biological activities of *S. alata* extracts and exploring their potential as sources for new acaricidal agents.

Anthraquinones, such as rhein identified in *Senna alata*, are noted for their anti-mite properties (Phongpaichit *et al*., 2004). Rhein has been documented for its activities against lepidopteran pests (Duraipandiyan *et al*., 2011) and human cancer cells through mechanisms like apoptosis (Chang *et al*., 2012). Based on these previous studies reporting the effect of anthraquinone against several arthropods, the acaricidal efficacy observed in this study may be attributed to anthraquinones present in *S. alata* leaf extract. However, this can be concluded with further *in vitro* and *in vivo* studies using only anthraquinone compounds against mites.

In conclusion, this study underscores the potential of *S. alata* leaf extract as a natural acaricide. It promotes sustainable alternatives to synthetic acaricides, supporting safer pest management practices in rabbit farming and aligning with animal welfare concerns. However, further research is required to investigate the long-term effects of frequent applications, the possible emergence of mite resistance, and the environmental impacts of these treatments. Furthermore, refining extraction techniques and solvents may improve the stability and efficacy of bioactive chemicals such as anthraquinones, which is thought to contribute to the reported acaricidal effects.

CONCLUSIONS AND RECOMMENDATIONS

This research underscores the efficacy of Senna alata leaf extract as a natural acaricide, presenting a sustainable alternative to synthetic solutions. Its application promotes safer pest management in rabbit farming while resolving animal welfare issues. Additional research is necessary to identify further bioactive chemicals, examine long-term benefits, and assess the danger of resistance development in mite populations.

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NOVELTY STATEMENTS

This research is the first to comprehensively evaluate the in vitro and in vivo acaricidal activity of *Senna alata* leaf extract against Psoroptes cuniculi in rabbits. Our data indicate that *S. alata* may serve as a viable natural alternative to synthetic acaricides, evidenced by substantial reductions in mite mortality and infestation. This study enhances sustainable pest management approaches in rabbit farming, simultaneously tackling issues of chemical resistance and animal welfare. Moreover, our findings facilitate additional investigation into bioactive substances and their modes of action against parasitic mites.

AUTHOR'S CONTRIBUTIONS

Nur Athiqah designed the study and drafted the manuscript, Nur Elisa conducted the *in vitro* study, Lananan helped in data analysis for in *vitro* study, Muhammad Erfan Faiq conducted the *in vivo* study, Ghazali and Chai helped in data analysis for *in vivo* study.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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