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Development and Evaluation of a Stable Topical Cream Formulated with *Annona squamosa* Seed Extract as a Natural Pediculosis Agent

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Abstract

Head lice infestation (*Pediculosis capitis*) remains a global public health concern, exacerbated by growing resistance to conventional pediculicides such as permethrin and malathion. This study aimed to evaluate the pediculicidal activity and formulation stability of a topical cream containing *Annona squamosa* (sugar apple or srikaya) seed extract as a natural alternative for treating pediculosis. The ethanolic extract of *A. squamosa* seeds was obtained through maceration, producing a 10.005% yield. Pediculicidal assays were conducted using various extract concentrations (5%, 7.5%, and 10%), followed by formulation of oil-in-water creams with extract concentrations of 7.5%, 10%, and 12.5%. Physical stability tests included assessments of viscosity, pH, spreadability, adhesion, and homogeneity. Results showed a dose–response relationship, with lice mortality increasing from 60% at 5% extract to 87% at 10%. Extract. The formulated creams demonstrated high efficacy—86% to 96% mortality—comparable to 1% permethrin. All formulations maintained acceptable physicochemical properties (pH 4.8–5.5, viscosity within 27,000–47,000 cps) and remained stable after accelerated storage. These findings indicate that *A. squamosa* seed extract is a potent pediculicidal agent that can be effectively incorporated into a stable topical formulation. The study supports the potential of *A. squamosa* as a safe, sustainable, and plant-based alternative for managing pediculosis while addressing the challenge of chemical resistance.

Keywords: *Annona squamosa*; *Pediculosis capitis*; topical cream formulation; head lice control.

INTRODUCTION

Pediculosis capitis, commonly referred to as head lice infestation, remains a significant public health concern, particularly among school-aged children. The World Health Organization (WHO) reports that the prevalence of head lice in developing countries ranges from 10-20% in children, with higher rates in rural areas and communities with poor sanitation. Despite the use of conventional pediculicides such as permethrin and malathion, the increasing resistance of lice to these chemicals has reduced their effectiveness, prompting the need for alternative treatments. Abbasi et al. (2022) and Chen et al. (2025) emphasize this growing challenge, underscoring the importance of exploring plant-based solutions. Recent studies suggest that plant-derived compounds can provide safer and more effective alternatives to conventional chemical treatments (Chen et al., 2025).

Several plant extracts and essential oils have shown significant pediculicidal and ovicidal properties. For example, essential oils from tea tree, neem, and coconut have demonstrated insecticidal effects against lice and their eggs. Research by Candy et al.,~~,~~ (2020) and Shailajan et al. (2013) supports the effectiveness of plant extracts, such as those from *Ageratum conyzoides* and *Cinnamomum porphyrium*, in reducing lice populations. Additionally, Campli et al. (2012) found that combinations of tea tree oil and nerolidol were more effective than conventional treatments. These findings highlight the potential of plant-based remedies to address the challenge of lice resistance to traditional treatments. Although physical agents like dimethicone have been explored, plant-based solutions generally offer greater efficacy and availability (Burgess el al., 2013; Kalari et al., 2019).

The development of resistance to conventional pediculicides remains a key issue in managing pediculosis. Diamantis et al. (2009) stress the need for alternatives that can avoid resistance development. Plant-based treatments offer a diverse range of mechanisms of action. Plants such as Melia azedarach, Ageratum conyzoides, and Tinospora crispa have proven effective with lower resistance risk, acting through mechanisms including ovicidal effects and disruption of lice

metabolism (Jayaseelan et al., 2011; Toloza et al., 2010). These findings underscore the importance of conducting further research into ethnomedicinal plants for the treatment of pediculosis.

Annona squamosa L. (Sugar apple or Srijaya) is widely used in traditional medicine, especially in tropical and subtropical regions. The seeds and fruit of this plant have long been recognized for their therapeutic properties. Previous studies have highlighted the antimicrobial potential of Annona squamosa, with ethanol extracts from its leaves shown to be effective against pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Dewangga et al., 2019; Goh et al., 2024). Furthermore, its antioxidant and anti-inflammatory properties make it a promising candidate for further exploration in the treatment of pediculosis (Ibrahim el al., 2024). The seeds, traditionally used for treating infections, also exhibit significant therapeutic potential (Maji, 2016).

With concerns over the use of conventional chemicals leading to resistance and side effects, a topical cream formulation based on *Annona squamosa* seed extract presents an appealing alternative. Topical creams offer advantages such as ease of application, better absorption, and uniform distribution on the scalp. They can also enhance comfort and provide long-term protection against lice infestations (Khairi et al., 2018). Additionally, combining natural active ingredients in cream formulations can improve efficacy while reducing irritation (Papa et al., 2023). Given these benefits, a cream based on *Annona squamosa* seed extract is expected to be a safer and more effective solution for managing pediculosis, particularly in the context of chemical resistance.

The primary objective of this study is to evaluate the activity and effectiveness of a stable topical cream derived from *Annona squamosa* seed extract as a potential treatment for pediculosis. Additionally, the study will assess the stability of the cream. This research aims to fill a gap in the literature regarding Annona squamosa's effectiveness as a pediculicidal agent, contributing to the search for alternative treatments to combat lice resistance.

MATERIALS AND METHODS

Materials Used

The materials used in this study include aluminum foil, stirring rods, a blender (Philips®), petri dishes, measuring cylinders (Iwaki®), beaker glasses (Iwaki®), rotary evaporator (R-100 with cold trap buchi), analytical balance (FS-AR210 (INT-CAL)), cream containers, pH meter (Laqua), dropper pipettes, filter paper, viscometer (Brookfield®), homogenizer (WiseStir®), and hot plate (DLAB). The materials used in the study include srikaya seed extract, ethanol 96% (Namaste), cetyl alcohol (Intraco), DMDM hydantoin (Intraco), isopropyl myristate (Intraco), phenoxyethanol (Intraco), cetrimonium chloride (Almega), steareth-20 (Almega), sodium metabisulfite (Meta), distilled water (One Med®), DMSO (EMSURE®), adult head lice, and Peditox®/permethrin 1% (PT. Combiphar).

Sample Extraction

The powder from the srikaya seeds was extracted using the maceration method. The extraction was carried out with a ratio of 1:5, immersing 1 kg of srikaya seed powder in 5000 mL of 96% ethanol for three days. After immersion, the mixture was filtered, and the filtrate was collected. The residue was remacerated, and the extract was concentrated by evaporating the solvent using a vacuum rotary evaporator at 50°C until a thick extract was obtained. The extract was then air-dried until a dry extract was achieved (Maji, 2016)

Antipediculosis Activity Test of Srikaya Seed Extract

The activity of the srikaya seed extract was tested using three concentrations: 5%, 7.5%, and 10%. The positive control used Peditox/permethrin 1%, while the negative control used DMSO. Five petri dishes and filter paper sized to fit the petri dishes were prepared, ensuring the bottom of the petri dishes was completely covered by the filter paper. 1.5 mL of each concentration of the extract and the controls was applied evenly on the surface of the petri dishes, which were lined with filter paper. Ten adult lice were placed in each petri dish. The movement of the lice was observed every 5 minutes for 2 hours (Shailajan et al., 2013). The lice were examined under a magnifying glass, and any signs of vital life, such as antenna movement or leg movement, were considered indicators that the lice were alive. Lice were considered dead if no vital signs were observed (Torre et al., 2017).

Formulation of Srikaya Seed Extract

Table 1. Formulation of Hair Cream Preparation.

Ingredient	Function	Concentration (% b/v)			
		F0	F1	F2	F3
Srikaya seed extract	Active ingredient	0	7,5	10	12,5
Cetyl alcohol	Thickener	5	5	5	5
Isopropyl myristate	Emolient	5	5	5	5
Phenoxyethanol	Preservative	1	1	1	1
DMDM Hydantoin	Preservative	0,5	0,5	0,5	0,5
Cetrimonium chloride	Conditioning	4	4	4	4
Steareth-20	Emulsifier	2	2	2	2
Sodium Metabisulfite	Antioxidant	0,10	0,10	0,10	0,10
Aquadest	Carrier	ad 100	ad 100	ad 100	ad 100

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration.

Preparation of Topical Cream Formulation from Srikaya Seed Extract

Each ingredient of the oil phase (cetyl alcohol, isopropyl myristate, steareth-20, and phenoxyethanol) was weighed and melted at a temperature of 75-80°C, starting from the highest melting point material. The water phase was heated to 75°C (DMDM hydantoin, sodium metabisulfite, cetrimonium chloride, and aquadest). After the oil phase was melted, it was added to the water phase and homogenized using a homogenizer until a cream base was formed (García et al., 2023; Pinto et al., 2021). The srikaya seed extract was then added according to the formulation (F1, F2, F3, except F0 = formula without extract). The mixture was homogenized until uniform, then placed into containers and sealed.

Stability Testing of the Formulation

The formulation was stored at 40°C for 24 hours. This experiment was conducted over six cycles, with evaluations conducted at the beginning and end of each cycle (Nešić et al., 2019). The parameters assessed were as follows:

- **Organoleptic Test**: The organoleptic evaluation involved observing the appearance, color, and aroma of each formulation (Humaira et al., 2025).
- **Homogeneity Test**: A sample of each cream formulation was applied to an object glass and examined for the presence of coarse particles or clumps. A good cream formulation should be free from coarse particles or clumps (Tania et al., 2022).
- **Viscosity Test**: A 30 g sample of cream was measured using a Brookfield viscometer with spindle number 64 set at 6 rpm. The ideal viscosity range for a good cream formulation is between 2,000-50,000 cps (Wangpradit et al., 2022).
- **pH Test**: A 30 g sample of cream was tested by immersing a pH meter into the cream, and the reading was taken after a brief waiting period. The optimal pH range for topical formulations is 4.5-6.5 (Lyu, 2024).
- **Spreadability Test**: A 0.5 g sample of cream was placed between two glass plates. Weights of 50 g, 100 g, and 200 g were applied sequentially, and the diameter of the spread was measured after 1 minute. The ideal spreadability value is between 5-7 cm (Parlapanska, 2024).
- **Adhesion Test**: A 0.25 g sample of cream was placed on an object glass and covered with a glass slide. A 250 g weight was added for 5 minutes, and then the object glass was placed in the test apparatus. A 50 g weight was applied, and the time taken for the cream to detach from the glass was recorded (Khairi et al., 2025).
- **Cream Type Test**: The type of cream was determined by applying 1 g of the formulation evenly onto an object glass and adding methylene blue drops while stirring. If the methylene blue dissolved evenly, the cream was identified as an oil-in-water emulsion type (Nur et al., 2025).

Effectiveness Testing of Srikaya Seed Extract Topical Cream as Antipediculosis

The effectiveness test aimed to ensure that the cream formulation produces the expected outcome as an antipediculosis treatment. For the procedure, five petri dishes were prepared, each lined with filter paper that completely covered the bottom of the petri dish. 1.5 mL of each cream formulation (F1, F2, F3), negative control (F0), and positive control (Peditox/permethrin 1%) was applied evenly on the surface of the petri dish lined with filter paper. Ten adult head lice were placed into each petri dish. The movement of the lice was observed every 5 minutes for a total duration of 2 hours (Shailajan et al., 2013). The lice were examined under a magnifying glass, and any signs of vital activity, such as movement of the antennae or legs, were considered indicators that the lice were alive. Lice were considered dead if no vital signs were observed (Torre et al., 2017).

RESULTS AND DISCUSSION

Yield of *Annona squamosa* Seed Extract

The maceration extraction of *Annona squamosa* seeds using 96% ethanol produced a yield of 10.005% (Table 2). This result reflects a high extraction efficiency considering that ethanol is a semi-polar solvent capable of dissolving a wide range of bioactive compounds such as alkaloids, flavonoids, and acetogenins, which are abundant in *A. squamosa* seeds (Maji, 2016). Acetogenins, in particular, are known for their potent insecticidal properties due to their ability to inhibit mitochondrial electron transport, leading to energy metabolism disruption in insects (Kazman J., 2022).

Table 2. Yield Percentage of *Annona squamosa* Seed Extract.

Sample	Solvent	Weight of Simplicia (gr)	Weight of Extract (gr)	Yield (%)
Annona squamosa seeds	Ethanol 96%	1000	101.05	10.005

Comparable yields (8–11%) have been reported by Dewangga A., (2019) for ethanolic extracts of *A. squamosa* leaves, indicating that both leaves and seeds are promising raw materials for pharmaceutical applications. These findings reaffirm the suitability of ethanol as a safe and effective extraction solvent for isolating phytoconstituents from *A. squamosa*, aligning with the growing emphasis on green extraction technologies (Chen et al., 2025).

Pediculicidal Activity of *Annona squamosa* Seed Extract

The pediculicidal activity of *A. squamosa* seed extract exhibited a concentration-dependent increase in mortality of *Pediculus humanus capitis* (Table 3). The extract at 5% concentration produced 60% mortality, which increased to 80% at 7.5% and 87% at 10%. These results were significantly different ($p < 0.05$) from the negative control (0%) and approached the efficacy of the positive control, 1% permethrin (100%).

Table 3. Pediculicidal Activity of *Annona squamosa* Seed Extract.

Sample	Mean Heal Lice Mortility ± SD	Mortality (%)
A. squamosa seed extract 5%	6.0 ± 1.0	60^{a}
A. squamosa seed extract 7.5%	8.0 ± 1.0	80^{b}
A. squamosa seed extract 10%	8.7 ± 0.6	87^{b}
Positive control (+)	10.0 ± 0.0	100^{b}
Negative control (-)	0.0 ± 0.0	0^{c}

^{a,b,c} Indicate significant differences according to LSD analysis (p<0,05,n=3).

This strong pediculicidal activity supports the hypothesis that *A. squamosa* contains bioactive compounds with potent insecticidal mechanisms. The observed efficacy is likely due to acetogenins and alkaloids that interfere with mitochondrial function, causing respiratory arrest and ultimately leading to the death of lice (Kazman J., 2022). Flavonoids may also contribute to this activity by penetrating the cuticle and destabilizing membrane proteins.

These findings are consistent with reports of other botanical pediculicides such as *Ageratum conyzoides* and *Cinnamomum porphyrium*, which demonstrated similar insecticidal properties (Shailajan et al., 2013; Candy et al., 2020). Moreover, the extract's performance parallels the combination of tea tree oil and nerolidol, which achieved 90% mortality in resistant lice populations (Campli et al., 2012). The similarity in efficacy underscores the potential of *A. squamosa* as a viable botanical alternative to neurotoxic pediculicides that are increasingly losing effectiveness due to resistance (Abbasi et al., 2022; Chen et al., 2025).

Physicochemical Characterization of the Cream Formulation

Organoleptic and Homogeneity Evaluation

The organoleptic analysis revealed stable physical characteristics across all formulations before and after accelerated storage (Table 4). The color ranged from white (F0) to light brown and brown (F1–F3), with a characteristic herbal odor corresponding to the extract concentration. All formulations were semi-solid and homogeneous, with no visible aggregates (Table 5).

Table 4. Organoleptic Observations Before and After Accelerated Storage.

Formula	Replicate	Before accelerated storage			After accelerated storage		
		Color	Odor	Consistency	Color	Odor	Consistency
F0	R1	White	Odorless	Semisolid	White	Odorless	Semisolid
	R2	White	Odorless	Semisolid	White	Odorless	Semisolid
	R3	White	Odorless	Semisolid	White	Odorless	Semisolid
F1	R1	Light brown	Charecteristic	Semisolid	Light brown	Charecteristic	Semisolid
	R2	Light brown	Charecteristic	Semisolid	Light brown	Charecteristic	Semisolid
	R3	Light brown	Charecteristic	Semisolid	Light brown	Charecteristic	Semisolid
F2	R1	Light brown	Charecteristic	Semisolid	Light brown	Charecteristic	Semisolid
	R2	Light brown	Charecteristic	Semisolid	Light brown	Charecteristic	Semisolid
	R3	Light brown	Charecteristic	Semisolid	Light brown	Charecteristic	Semisolid
F3	R1	Brown	Charecteristic	Semisolid	Brown	Charecteristic	Semisolid
	R2	Brown	Charecteristic	Semisolid	Brown	Charecteristic	Semisolid
	R3	Brown	Charecteristic	Semisolid	Brown	Charecteristic	Semisolid

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration.

Such consistency reflects proper emulsification and dispersion of active ingredients. The color stability also indicates minimal oxidative degradation of phenolic compounds during storage, confirming good chemical stability (Pinto et al., 2021). Homogeneity is particularly critical for ensuring dose uniformity and user acceptability in topical applications (Parvanescu et al., 2025).

Table 5. Homogeneity Test Results Before and After Accelerated Storage.

Formula	Replicate	Before Accelerated Storage	After Accelerated Storage
F0	R1	Homogeneous	Homogeneous
	R2	Homogeneous	Homogeneous
	R3	Homogeneous	Homogeneous
F1	R1	Homogeneous	Homogeneous
	R2	Homogeneous	Homogeneous
	R3	Homogeneous	Homogeneous
F2	R1	Homogeneous	Homogeneous
	R2	Homogeneous	Homogeneous
	R3	Homogeneous	Homogeneous
F3	R1	Homogeneous	Homogeneous
	R2	Homogeneous	Homogeneous
	R3	Homogeneous	Homogeneous

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration.

Viscosity

Viscosity values increased with higher extract concentrations: from 27,500 cps (F0) to 47,666 cps (F3) before storage (Table 6). After accelerated storage, viscosity slightly decreased but remained within the acceptable range (28,166–41,333 cps). According to Wangpradit et al. (2022), an ideal viscosity range for cosmetic creams is 2,000–50,000 cps, confirming the formulations' suitability.

Table 6. Viscosity Measurement Results Before and After Accelerated Storage.

Formula	Mean ± SD	
	Before Storage (cps)	After Storage (cps)
F0	27500 ± 0.25	28166 ± 0.20
F1	29500 ± 0.30	28500 ± 0.25
F2	40000 ± 0.28	34000 ± 0.23
F3	47666 ± 0.55	41333 ± 0.50

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration.

The increased viscosity in formulations containing extract suggests molecular interactions between polyphenols and emulsion components, reinforcing the internal structure of the cream (Naeimifar et al., 2023). The minor reduction in viscosity after storage (<10%) indicates strong thermodynamic stability of the oil-in-water (O/W) emulsion, which was maintained by the presence of *steareth-20* and *cetyl alcohol* as emulsifier and stabilizer, respectively (Guzmán et al., 2022; Sharkawy et al., 2020).

pH Stability

The pH values of all formulations ranged from 4.8 to 5.5 (Table 7), which remained within the physiological range for scalp compatibility (4.5–6.5). Slight decreases in pH after storage are attributed to mild oxidation of phenolic compounds, a common occurrence in natural formulations (Saleem et al., 2022).

Table 7. pH Measurement Results Before and After Accelerated Storage.

Formula	Mean ± SD	
	Before Storage	Before Storage
F0	5.49 ± 0.15	4.91 ± 0.07
F1	4.87 ± 0.08	4.82 ± 0.06
F2	4.98 ± 0.06	4.89 ± 0.08
F3	5.11 ± 0.08	5.01 ± 0.08

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration

A stable pH is essential to ensure the chemical integrity of the active compounds and user comfort, thereby preventing irritation or alteration of the skin microbiota (Namjoshi et al., 2020). The maintained pH in all extract-containing creams demonstrates that the buffer system and formulation design effectively mitigated degradation reactions.

Spreadability and Adhesion

Spreadability and adhesion tests (Tables 8 and 9) revealed satisfactory results across all formulations. Adhesion values ranged between 97–99 seconds, indicating sufficient retention on the scalp surface for therapeutic action. Spreadability values of 5.3–5.7 cm were consistent with the optimal standard (5–7 cm) for easy application and uniform distribution (Parlapanska, 2024).

Table 8. Adhesion Test Results Before and After Accelerated Storage.

Formula	Mean ± SD	
	Before Storage (s)	Before Storage (s)
F0	97.67 ± 1.53	98.33 ± 1.15
F1	98.33 ± 1.15	98.67 ± 0.58
F2	98.67 ± 0.58	99 ± 0.0
F3	98.33 ± 1.15	99 ± 0.0

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration

The inverse relationship between viscosity and spreadability, as observed here, aligns with rheological principles of pseudoplastic flow typical of topical emulsions (Hemalatha et al., 2022). These findings suggest that the cream's texture remains acceptable even with increased extract content, ensuring both efficacy and user compliance.

Table 9. Spreadability Test Results Before and After Accelerated Storage.

Formula	Mean ± SD	
	Before Storage (cm)	Before Storage (cm)
F0	5.2 ± 0.025	5.70 ± 0.07
F1	5.53 ± 0.03	5.59 ± 0.06
F2	5.45 ± 0.025	5.52 ± 0.08
F3	5.34 ± 0.04	5.47 ± 0.08

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration

Cream Type

The dye test confirmed all formulations as oil-in-water (O/W) type before and after storage (Table 10). O/W creams are preferred for pediculicidal applications due to their light texture, ease of rinsing, and lower risk of scalp irritation (Namjoshi et al., 2020). This emulsion type also enhances the release and absorption of hydrophobic active compounds, supporting the delivery efficiency of acetogenins and flavonoids from the *A. squamosa* extract (García et al., 2023).

Table 10. Cream Type Determination Before and After Accelerate Storage.

Formula	Replicate	Before accelerated Storage	After Accelerated Storage
F0	R1	Oil-in-water (O/W)	Oil-in-water (O/W)
	R2	Oil-in-water (O/W)	Oil-in-water (O/W)
	R3	Oil-in-water (O/W)	Oil-in-water (O/W)
F1	R1	Oil-in-water (O/W)	Oil-in-water (O/W)
	R2	Oil-in-water (O/W)	Oil-in-water (O/W)
	R3	Oil-in-water (O/W)	Oil-in-water (O/W)
F2	R1	Oil-in-water (O/W)	Oil-in-water (O/W)
	R2	Oil-in-water (O/W)	Oil-in-water (O/W)
	R3	Oil-in-water (O/W)	Oil-in-water (O/W)
F3	R1	Oil-in-water (O/W)	Oil-in-water (O/W)
	R2	Oil-in-water (O/W)	Oil-in-water (O/W)
	R3	Oil-in-water (O/W)	Oil-in-water (O/W)

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration.

Effectiveness of the *Annona squamosa* Seed Cream Against Head Lice

The topical cream containing *A. squamosa* extract exhibited strong pediculicidal efficacy, increasing with extract concentration (Table 11). F0 (placebo) showed no mortality, while F1 (7.5%) caused 86%, F2 (10%) 93%, and F3 (12.5%) 96% mortality, comparable to permethrin (100%). Statistical analysis confirmed significant differences ($p < 0.05$) between the negative control and all extract-containing formulations.

Table 11. Effectiveness Test Results of Annona squamosa Seed Extract.

Formula	Mean Head Lice Mortality ± SD	Mortality (%)
F0	0.0 ± 0.0	0a
F1	8.67 ± 0.58	86[b]
F2	9.33 ± 0.58	93[b]
F3	9.67 ± 0.58	96[b]
Positive Control (+)	10 ± 0.0	100[b]

[a,b] Indicate significant differences according to LSD analysis (p<0,05,n=3)

F0: Cream formulation without extract; F1: Cream formulation with 7.5% extract concentration; F2: Cream formulation with 10% extract concentration; F3: Cream formulation with 12.5% extract concentration

These results verify that the bioactive compounds remain stable and effective after formulation. The primary mechanism is likely mitochondrial respiration inhibition by acetogenins, resulting in paralysis and death of lice (Kazman J., 2022). This finding is consistent with previous reports on other plant-based insecticidal agents such as *Melia azedarach* and *tea tree oil* (Rossini et al., 2007; Toloza et al., 2010). In comparison with herbal mixtures such as *Illicium verum* and coconut oil (Armiyanti et al., 2020), the *A. squamosa*-based cream demonstrates equivalent or superior efficacy while offering greater formulation stability. Furthermore, its physicochemical robustness across multiple parameters (pH, viscosity, spreadability) aligns with the principles of *Quality by Design* (QbD), ensuring predictable performance and safety (Namjoshi et al., 2020).

The study thus establishes the *A. squamosa* seed cream as a competitive botanical alternative to conventional pediculicides. Its dual functionality—pediculicidal and antimicrobial—further enhances its therapeutic potential by reducing secondary scalp infections (Dewangga A., 2019; Goh C., 2024). The present findings contribute to the global discourse on sustainable, plant-based approaches to managing pediculosis. Unlike neurotoxic pediculicides such as permethrin and malathion, which face escalating resistance issues (Abbasi et al., 2022; Burgess et al., 2013), plant-derived formulations act through multiple biochemical pathways, making resistance less likely (Jayaseelan, 2011).

The successful formulation of a stable and effective cream exemplifies how ethnomedicinal knowledge can be integrated into modern pharmaceutical design under the Quality Target Product Profile (QTPP) framework. Future studies should investigate nanoscale delivery systems—such as nanoemulsions or Pickering emulsions—to enhance bioavailability and stability (Guzmán et al., 2022; Mascarenhas-Melo et al., 2023). Furthermore, the strong antioxidant profile of *A. squamosa* may complement its pediculicidal activity by mitigating oxidative stress on the scalp, promoting scalp health and recovery (Ibrahim et al., 2024; Ma et al., 2017). This multifaceted therapeutic profile underscores the potential of *A. squamosa* for developing next-generation herbal pediculicidal formulations.

CONCLUSIONS

This study demonstrates that *Annona squamosa* seed extract is an effective pediculicidal agent that remains active and stable when formulated into a topical cream. The extraction process yielded 10.005%, indicating the presence of sufficient bioactive constituents for product development. Biological assays revealed a clear dose–response relationship, with head lice mortality increasing from 60% at 5% concentration to 80% at 7.5% and 87% at 10%, showing significant differences compared with the negative control. Once formulated, the cream exhibited high pediculicidal activity—86% at 7.5%, 93% at 10%, and 96% at 12.5%—approaching the efficacy of the standard permethrin (100%). From a formulation standpoint, the cream maintained acceptable physicochemical characteristics after accelerated storage. Its viscosity remained within the ideal range with minimal reduction, pH values (4.8–5.5) were compatible with scalp physiology, adhesion ranged from 97–99 seconds, and spreadability (approximately 5.3–5.7 cm) supported ease of application. All formulations were confirmed to be oil-in-water emulsions, making them suitable for therapeutic scalp use and easy rinsing. These findings suggest that *A. squamosa*-based cream represents a promising natural alternative for addressing pediculicide resistance while providing a safe and stable topical formulation. The study contributes to existing knowledge by demonstrating consistent efficacy and formulation stability of *A. squamosa* seed extract in a topical delivery system. Future research should include controlled clinical trials in target populations, evaluation of ovicidal activity, repeated-use safety assessments, long-term stability studies, and optimization of delivery systems such as nanoemulsions to enhance bioavailability.

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