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FORMULATION AND EVALUATION OF HERBAL ANTI-LICE SHAMPOO FROM *ANNONA RETICULATA* (FAMILY: *ANNONACEAE*) LEAF EXTRACT

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Abstract:
Head lice infestation is a common parasitic condition that can cause itching, scalp irritation, and discomfort. The increasing concerns associated with synthetic pediculicides have encouraged the development of safer herbal alternatives. This study aimed to formulate and evaluate an herbal anti-lice shampoo containing ethanolic leaf extract of *Annona reticulata* (family *Annonaceae*). The leaves were extracted by maceration using ethanol and subjected to qualitative phytochemical screening. The extract contained several phytoconstituent groups, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, phenolic compounds, and carbohydrates. The herbal shampoo was prepared using suitable surfactants and excipients and evaluated for various physicochemical parameters. The formulation exhibited a pH of 5, density and specific gravity of 1.025 g/mL, satisfactory viscosity and foaming properties, and acceptable physical characteristics. Skin irritancy testing showed no visible redness or swelling during the observation period. In the anti-lice evaluation, the formulation demonstrated pediculicidal activity, with complete mortality of lice observed within 3 minutes, showing faster activity than the marketed herbal shampoo used for comparison. The findings indicate that *Annona reticulata* leaf extract has potential for incorporation into herbal anti-lice shampoo formulations.

Keywords: *Annona reticulata*; Herbal shampoo; Head lice; Pediculicidal activity; Phytochemicals; Leaf extract.

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INTRODUCTION

Head lice (*Pediculushumanuscapitis*) infestation is a prevalent parasitic condition, particularly among school-aged children, leading to itching, scalp irritation, and secondary infections. Synthetic pediculicides such as permethrin and malathion are commonly used but are associated with side effects, resistance development, and environmental concerns [1]. Hence, the exploration of safe and effective herbal alternatives is gaining interest. *Annona reticulata*, a plant of the family *Annonaceae*, is traditionally used in ethnomedicine for its insecticidal, antimicrobial, anthelmintic, anti-inflammatory, antipyretic, and antiparasitic properties. Its leaves are known to contain bioactive compounds such as acetogenins, alkaloids, glycosides, tannins, phenols, and flavonoids, which may contribute to anti-lice properties. Tree 3-8 meters tall, cylindrical stem with numerous white lenticels, young leaves and stems densely covered with yellowish hairs. Leaf blade elliptical, oblong or lanceolate, covered with minute yellowish hairs, especially on veins; apex acute, the base obtuse or rounded, margin entire, and petioles 0.5- 1.5 cm long [2, 3]. Previous studies have reported promising pediculicidal activity of several plant-based shampoos, supporting shampoo as a suitable topical vehicle for delivering plant-derived active constituents to the scalp and hair. *Annona reticulata* (*Annonaceae*) is traditionally recognized for its medicinal properties, andits leaves contain bioactive phytoconstituents with reported insecticidal potential [4].

MATERIAL AND METHODS

Authentication of Plant Material

The plant material used in the present study was authenticated by Dr. Rogimon P. Thomas, Ph.D., Head, Department of Botany, CMS College Kottayam (Autonomous), Kerala, India. The selected plant specimen was identified as *Annona reticulata L.*, belonging to the family *Annonaceae*. Authentication was carried out on 08 December 2025, and a Plant

Authentication Certificate was obtained and retained for reference.

Maceration

The leaves of *Annona reticulata* were collected from Ernakulam district, shade-dried, powdered, and passed through sieve No. 20. The powdered leaves (50 g) were subjected to maceration with ethanol (500 mL) in a sealed glass container for 24–72 h at room temperature, with occasional agitation. The extract was then filtered through Whatman filter paper and concentrated. The resulting extract was stored in a labeled, airtight container under cool and dark conditions until further use. The extraction yielded 4.5 g of dry extract [5].

Qualitative Phytochemical Evaluation of *Annona reticulata* Leaf Extract

The ethanolic leaf extract of *Annona reticulata* was subjected to qualitative phytochemical screening using standard procedures to identify the presence of various classes of bioactive components:

Preliminary Phytochemical Screening

Table 01: Phytochemical Evaluation

Sl. No.	Phytochemical Constituent	Test Performed	Positive Result/Inference
1	Alkaloids	Dragendorff's test	Orange precipitate indicates the presence of alkaloids
		Mayer's test	Cream-colored precipitate indicates the presence of alkaloids
2	Flavonoids	Alkaline reagent test	Yellow coloration that disappears upon addition of dilute acid indicates flavonoids
3	Tannins	Ferric chloride test	Blue-black or greenish-black coloration indicates tannins
4	Saponins	Froth test	Persistent foam formation indicates the presence of saponins
5	Glycosides	Keller–Killiani test	Reddish-brown layer at the interface indicates cardiac glycosides
6	Terpenoids	Salkowski's test	Reddish-brown coloration at the interface indicates terpenoids
7	Steroids	Liebermann–Burchard test	Green coloration indicates the presence of steroids
8	Phenolic compounds	Ferric chloride test	Deep blue or black coloration indicates phenolic compounds
9	Carbohydrates	Molisch's test	Violet ring at the interface indicates carbohydrates
10	Proteins and amino acids	Biuret test	Absence of violet coloration indicates the absence of proteins

FORMULATION

Ingredients and Procedure for each 100ml of formulation Ingredients

Table 02: Ingredients and Procedure

Ingredients	Quantity
Sodium lauryl sulphate paste	22 gm
Oleic acid	10gm
Triethanolamine	5gm
Extract (dried)	1.5 gm
Perfume	Quantity Sufficient
Preservative	Quantity Sufficient
Colour	Quantity Sufficient
Water	Quantity Sufficient to make up to 100 ml

Mix water, oleic acid, and sodium lauryl sulphate paste and heat to 60°C. Slowly add triethanolamine with continuous stirring; add extract (dried) dissolved in 5ml of water; make up with water to 100ml with continuous stirring. Add perfume; cool to 35 °C [4].

EVALUATION

To evaluate the prepared formulations, quality control tests, including visual assessment and physicochemical controls such as pH, density, and viscosity performed. This also assures the quality of products; specific tests for shampoo formulations, including the determination of dry residue and moisture content, surface tension, and detergency tests, should be carried out.

RESULTS

1.Percentage Yield

The percentage yield was found to be 9.6%w/w

2.Evaluation Test Results of Herbal Shampoo Preparation

The pH of the prepared shampoo was 5. This indicates that the shampoo is mildly acidic and suitable for use on the scalp and hair. A pH near the natural scalp pH helps in maintaining hair health and reduces irritation.

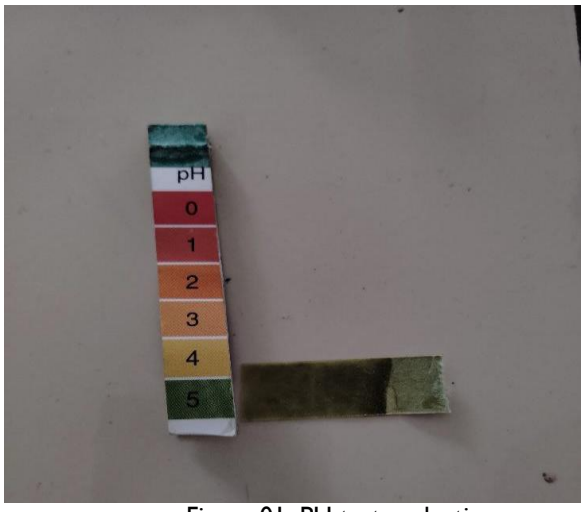


Figure 01: PH test evaluation

3. Density Determination

The density of the shampoo was found to be 1.025 g/mL. This value indicates good consistency and proper formulation characteristics of the shampoo preparation.

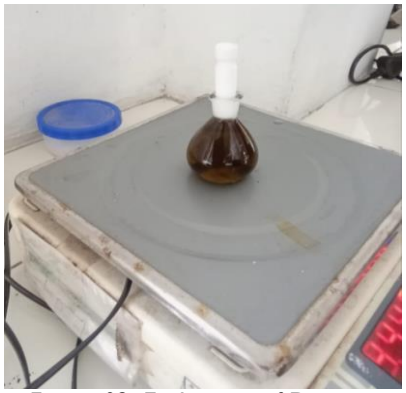


Figure 02: Evaluation of Density

4. Viscosity Test

The viscosity of the shampoo was determined using a viscometer with spindle number 64 at 1 rpm. The observed reading was 98.5% torque. This result indicates that the shampoo possesses adequate viscosity, which helps in easy application and spreading on hair.

5. Moisture Content/ Loss on Drying

The moisture content determined by the loss on drying method was found to be 40%. This indicates the percentage of volatile matter and water content present in the formulation.

Initial weight =2.5g

Final weight = 1.5g

Moisture content =Final weight – Initial weight / Initial weight *100 = 40%

6. Foam Test

The foaming ability of the shampoo was evaluated by measuring foam height at different time intervals.

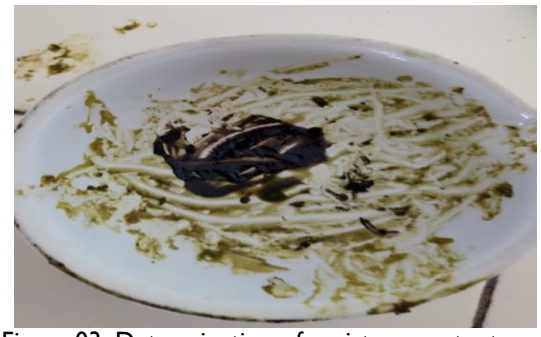


Figure 03: Determination of moisture content

7. Foam Stability Test

The shampoo exhibited good foaming properties initially. However, foam stability gradually decreased over time, indicating a reduction in the persistence of the foam.

Table 03: Foam Test Results

Time Interval	Foam Height
After 10 Min	10ml
After 20 Min	5ml

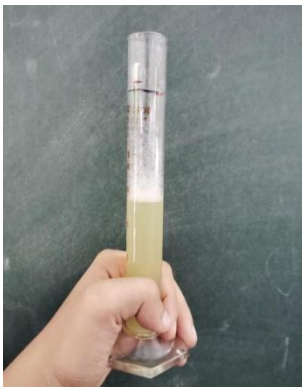


Figure 04: After10minutes, height=10ml.



Figure 05: After20minutes, height=5ml

8. Specific Gravity

The specific gravity of the shampoo formulation was determined to be 1.025, indicating that the formulation was slightly denser than water. This value suggests acceptable physical characteristics for a shampoo formulation.

Formula:

Specific gravity = weight of sample/weight of water

= 1.025gm/ml = 25/24.4

Therefore, the specific gravity of the shampoo was found to be 1.025 (dimensionless).

9. Skin Irritancy Test

A skin irritancy test was performed to evaluate the potential irritation caused by the shampoo formulation. The formulation was applied to pork skin, and the test area was observed for signs of irritation. After 24 hours, no visible redness, swelling, or other signs of irritation were observed. The observation period was extended to 72 hours, during which no redness or swelling was noted. These observations indicate that the formulation did not produce visible signs of irritation on the pork skin under the test conditions. However, this preliminary test alone does not establish the formulation's safety for human use.



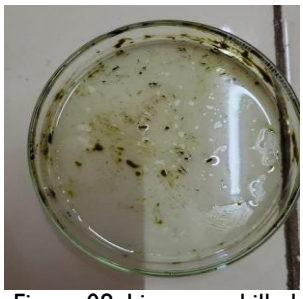
Figure 06: Starting

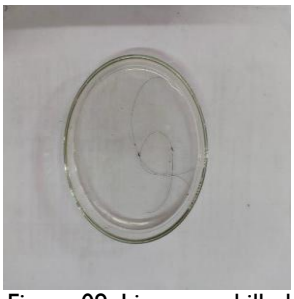


Figure 07: After 24 hours

10. Anti-Lice Activity

The anti-lice activity of the formulated shampoo was evaluated using live lice. The shampoo sample was applied to the lice, and their activity was monitored to assess the effectiveness of the formulation. The lice were observed to die within 3 minutes of application. The formulated shampoo demonstrated faster lice-killing activity than the marketed herbal anti-lice shampoo under the test conditions. These findings suggest that the formulation may possess promising anti-lice properties. However, further testing with appropriate controls and replicate experiments is required to confirm its efficacy.


Figure 08: Lice were killed Within 3 minutes.


Figure 09: Lice were killed Within 5 minutes

DISCUSSION

The present study aimed to formulate and evaluate an herbal anti-lice shampoo containing ethanolic leaf extract of *Annona reticulata*. Phytochemical screening of the extract revealed the presence of various phytoconstituents, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, phenols, and carbohydrates. These bioactive constituents may contribute to the potential pediculicidal activity of the formulation. The prepared shampoo exhibited acceptable physicochemical properties. The pH of 5 indicated that the formulation was mildly acidic and within the commonly preferred pH range for scalp-care products. The specific gravity was found to be 1.025, suggesting that the formulation was slightly denser than water. The viscosity indicated suitable consistency for convenient application, while the formulation demonstrated satisfactory foaming properties. However, foam stability gradually decreased over time.

The preliminary skin irritancy test performed on pork skin showed no visible redness or swelling during the observation period of up to 72 hours. These findings suggest that the formulation did not produce visible irritation under the specific test conditions. However, further safety evaluation is necessary before establishing its suitability for human use. The anti-lice activity evaluation demonstrated that the formulated shampoo killed lice within 3 minutes. This result indicated faster lice-killing activity than the marketed herbal anti-lice shampoo used for comparison under the experimental conditions. The observed activity may be associated with bioactive constituents present in the extract, potentially including acetogenins and other phytochemicals. Nevertheless, further controlled studies are required to identify the active compounds and confirm their contribution to the observed effect. Overall, the findings suggest that *Annona reticulata* leaf extract has potential for further investigation as an ingredient in herbal anti-lice shampoo formulations. Additional studies involving standardized testing, formulation optimization and comprehensive safety assessment are required to establish its efficacy and suitability for practical applications.

FUTURE PERSPECTIVES

- **Standardization:** Establish appropriate standardization parameters for *Annona reticulata* leaf extract and the developed shampoo to ensure consistent quality, reproducibility, and batch-to-batch uniformity.
- **Formulation optimization:** Apply a design-space approach to systematically optimize critical formulation variables, including extract concentration, surfactant levels, pH, and viscosity, within acceptable ranges.
- **Identification of bioactive constituents:** Investigate the phytochemical constituents responsible for pediculicidal activity using suitable analytical techniques.
- **Mechanistic studies:** Explore the possible mechanisms through which the extract affects lice survival and activity.
- **Comparative efficacy studies:** Conduct controlled experiments with appropriate controls and multiple replicates to compare the formulation with marketed anti-lice products.
- **Safety and stability assessment:** Evaluate the formulation's stability, potential toxicity, scalp compatibility, and safety using appropriate validated methods before considering human use or commercialization.

CONCLUSION

The present study successfully formulated and evaluated an herbal anti-lice shampoo containing ethanolic leaf extract of *Annona reticulata*. Phytochemical screening revealed the presence of several classes of phytoconstituents that may

contribute to the biological activity of the extract. The developed formulation exhibited acceptable physicochemical characteristics, including a pH of 5, a specific gravity of 1.025, suitable consistency, and satisfactory foaming ability. No visible redness or swelling was observed during the preliminary skin irritancy assessment on pork skin over a period of up to 72 hours. Furthermore, the anti-lice activity evaluation showed that the formulation killed lice within 3 minutes under the experimental conditions. These preliminary findings indicate that *Annona reticulata* leaf extract may be a promising candidate for further research and development of herbal anti-lice shampoo formulations. However, additional studies involving standardized efficacy testing, active constituent identification, comprehensive safety evaluation, and formulation stability assessment are necessary before its safety and effectiveness for human use can be established.

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1. Department of Botany, CMS College Kottayam (Autonomous), Kerala, India: Authentication of the plant material was carried out by Dr. Rogimon P. Thomas, Ph.D., Head, Department of Botany.
2. Nirmala College of Pharmacy, Muvattupuzha, Kerala, India: Conducted the experimental design, animal studies, and ex vivo studies related to the formulation and evaluation of the herbal anti-lice shampoo.

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