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Research Article

# Phytochemical Screening and Anti-Lice Activity of *Annona squamosa* Leaves

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## ABSTRACT

**Background:** *Annona squamosa* is a medicinal plant traditionally used for various therapeutic applications. The present study was conducted to evaluate the phytochemical constituents and anti-lice activity of the hydroalcoholic extract of *Annona squamosa* leaves. **Materials and Methods:** The phytochemical analysis revealed the presence of carbohydrates, proteins, tannins, glycosides, alkaloids, flavonoids, steroids, and saponins, while amino acids were absent. Tannins were found in abundant quantity, and flavonoids, glycosides, and saponins were present in high amounts. The extract showed dark greenish brown color, characteristic odor, sticky consistency, and 15% yield. Fluorescence analysis of leaf powder showed characteristic colour changes under visible and UV light, supporting its pharmacognostic identification. **Results and Discussion:** The anti-lice activity was evaluated against head lice using 2.5%, 5%, and 10% concentrations of extract, with 5% benzyl benzoate as standard. The hydroalcoholic extract exhibited significant concentration-dependent activity. The 10% extract showed the highest effect with 95% mortality and mean death time of 15.23±0.21 min, followed by 5% extract (80%) and 2.5% extract (58%). The standard showed 100% mortality within 14.47±0.38 min, while the control showed no activity. **Conclusion:** The hydroalcoholic extract of *Annona squamosa* leaves contained important bioactive phytochemicals and showed significant concentration-dependent anti-lice activity. The 10% extract produced 95% lice mortality, comparable to benzyl benzoate. These results support its traditional use as a natural pediculicidal agent. Further studies are needed to evaluate its safety, isolate active compounds, and develop effective formulations.

**Keywords:** *Annona squamosa*, Hydroalcoholic extract, Phytochemical screening, Anti-Lice activity, Head lice.

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## INTRODUCTION

Before the development of synthetic drugs, man depended on the therapeutic properties of medicinal plants. Some individuals value plants because of the old belief that they were made to give humans food, medicine, and other advantages (Davidson-Hunt, 200). Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for various functions, including, defence and protection against insects, fungi, diseases, and herbivorous mammals (Gershenzon and Ullah, 2022).

Medicinal plants are the major sources of numerous valuable chemicals and/or drugs. Over 1300 medicinal plants are used in European countries, and out of them, 90% are from wild sources (Gusain *et al.*, 2021).

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, against parasites (Newman and Cragg, 2020), and herbivorous mammals.

Drug research sometimes makes use of ethnobotany to search for pharmacologically active substances, and this approach has yielded hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are diverse, with most in four biochemical classes: alkaloids, glycosides, polyphenols, and terpenes. Few of these are scientifically confirmed as medicines or used in conventional medicine.

Medicinal plants are widely used as folk medicine in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. In many countries, there is little regulation of traditional medicine, but the

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WHO coordinates a network to encourage safe and rational use. The botanical herbal market has been criticized for being poorly regulated and containing placebo and pseudoscience products with no scientific research to support their medical claims. Medicinal plants face both general threats, such as climate change and habitat destruction, and the specific threat of over-collection to meet market demand (Ahn, 2017).

*Annona squamosa* is a small, well-branched tree or shrub from the family Annonaceae that bears edible fruits called sugar apples or sweetsops or custard apples. It tolerates a tropical lowland climate better than its relatives *Annona reticulata* and *Annona cherimola* helping make it the most widely cultivated of these species.

*Annona squamosa* is semi-(or late) deciduous, and 3 to 8 M (10 to 26 feet) tall, similar to soursop (*Annona muricata*). It is a native of tropical climate in the Americas, and Spanish traders aboard the Manila galleons docking in the Philippines brought it to Asia (Kral, 2019).

The fruit is spherical-conical, 5-10 cm (2-4 inches) in diameter and 6-10 cm (2+1⁄4-4 in) long, and weighing 100-240 g (3.5-8.5 ounces), with a thick rind composed of knobby segments. The colour is typically pale green through blue-green, with a deep pink blush in certain varieties, and typically has a bloom. It is unique among *Annona* fruits in being segmented; the segments tend to separate when ripe, exposing the innards.

The flesh is fragrant and sweet, creamy white through light yellow, and resembles and tastes like custard. The seeds are coated with the flesh, it is found adhering to 13 mm to 16 mm long (1⁄2 to 5⁄8 in) seeds forming individual segments arranged in a single layer around a conical core. It is soft, slightly grainy, and slippery. The hard, shiny seeds may number 20-40 or more per fruit and have a brown to black coat, although varieties exist that are almost seedless (Morton, 1987).

New varieties are also being developed in Taiwan and Hong Kong. The atemoya or "pineapple sugar-apple", a hybrid between the sugar-apple and the cherimoya, is popular in Taiwan, although it was first developed in the United States in 1908. The fruit is similar in sweetness to the sugar-apple, but has a very different taste. As its name suggests, it tastes like pineapple

## MATERIALS AND METHODS

### Assortment of Plant

The plant leaves of *Annona squamosa* were collected from Kodad regions of Suryapet Dist. T.G, the leaves were shade-dried, powdered and kept in an air-tight container. The end product was a powder and this powder was extracted.

### Extraction

150 g of dried powdered material of *Annona squamosa* leaves was mixed with 1000 mL of hydroalcoholic solvent in a 1:10 ratio in a conical flask. The mixture was subjected to sonication using an ultrasonic bath at 20-40 kHz for 30 min at room temperature. After sonication, the extract was filtered through Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator or water bath below 50°C to obtain the crude extract. The obtained extract was dried and stored in an airtight container for further studies. The percentage yield, color, and consistency were determined (Sudhakar *et al*., 2024).

### Phytochemical Screening

The extract obtained was subjected to qualitative chemical test for the identification of various chemical constituents.

### Fluorescence Analysis

When various chemicals and solvents are applied to plant material, the chemical components present display a phenomenon called fluorescence. The current work investigated the fluorescence of powdered *Lagerstroemia speciosa* (L.) leaves under UV and visible light/daylight settings following treatment with different agents and solvents. A little amount of dried and finely powdered material was treated with a variety of solvents, solutions, and freshly prepared acids. The powder sample was treated with acids such as concentrated $HNO_3$, HCl and $H_2SO_4$, and acetic acid in addition to alkaline solutions such as 10% aqueous NaOH and different solvents such as ammonia, distilled water, 5% $FeCl_3$, iodine, methanol, and ethanol. Their fluorescence in visible and ultraviolet light was studied (Kommu and Sivakumar, 2026).

### Evaluation of Anti-Lice Activity

Clean and dry Petri dishes were taken and labelled into the following groups:

- Control.
- Standard (5% Benzyl benzoate).
- 2.5% extract.
- 5% extract.
- 10% extract.

Whatman filter paper was placed inside each Petri dish. Approximately five live lice were transferred into each dish using forceps.

About 1.5 mL of the respective test solution was uniformly applied onto the filter paper in each Petri dish. The dishes were then covered and maintained at room temperature.

The lice were observed at regular time intervals for signs of paralysis and mortality. The time required for complete mortality of lice in each group was recorded in minutes. Lice showing no movement even after gentle probing with forceps were considered dead (Sudhakar *et al*., 2026).

## RESULTS

### Organoleptic results of extract

The observation results of Hydro alcoholic extract of *Annona squamosa* leaves were given below (Table 1 and Figure 1).

The preliminary phytochemical studies of hydroalcoholic extract were tested for different phytoconstituents such as carbohydrates, proteins, amino acids, tannins, glycosides, alkaloids, flavonoids, steroids, and saponins using standard procedures. The results obtained are shown in Table 2 and image of tests in Figure 2.

**Table 1: Organoleptic results of extracts.**

| Properties | Hydro alcoholic extract |
|---|---|
| Colour | Dark greenish brown |
| odour | Characteristic |
| Consistency | Sticky |
| % Yield | 15% |

### Fluorescence analysis

The fluorescence analysis of the leaves powder revealed characteristic colour changes under visible light, 254 nm, and 365 nm UV light after treatment with different chemical reagents. These fluorescence variations indicate the presence of different phytochemical constituents in the leaf material and serve as an important pharmacognostic parameter for the identification and authentication of the crude drug.

The untreated leaf powder showed a greenish brown colour in visible light, dark green fluorescence at 254 nm, and dark green/black fluorescence at 365 nm, indicating the natural fluorescence characteristics of plant pigments and other phytoconstituents. Treatment with distilled water produced black fluorescence

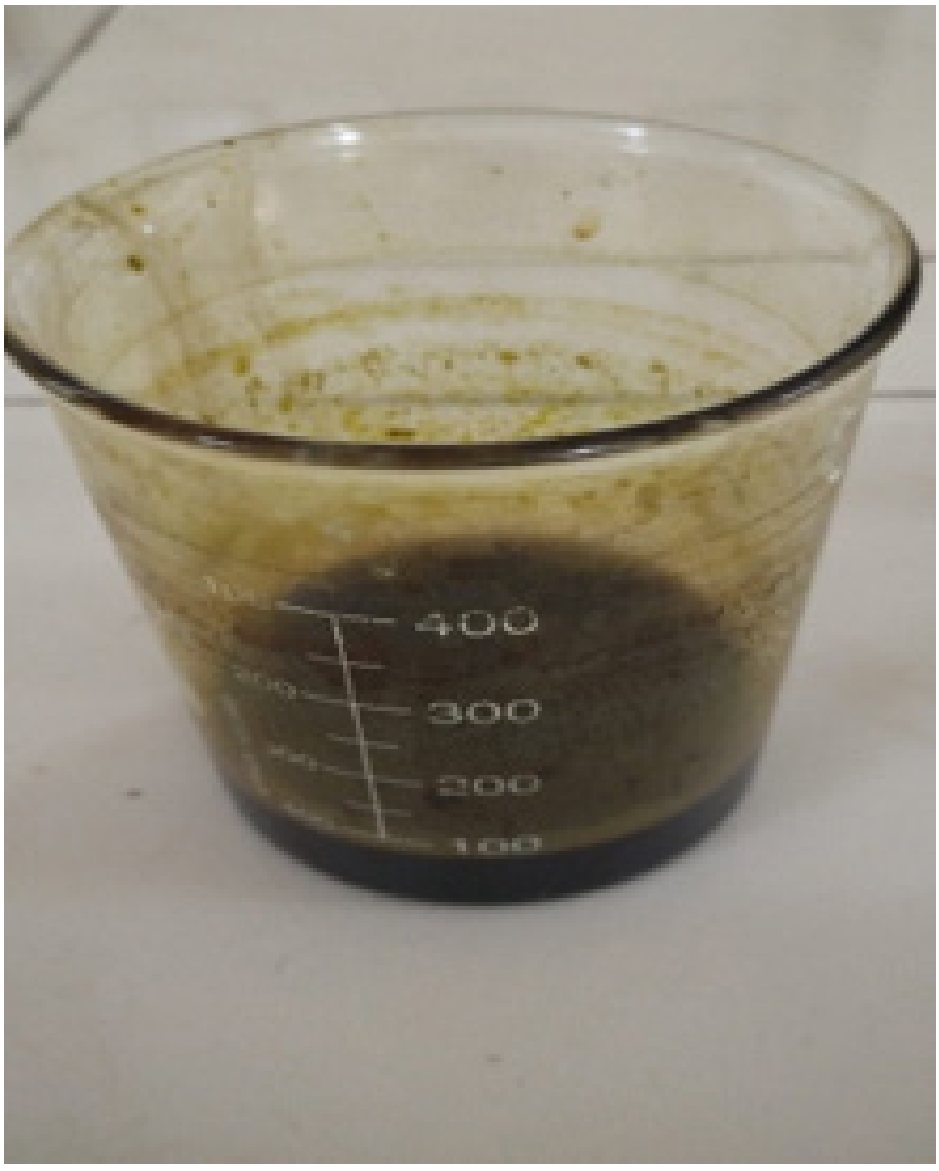


**Figure 1:** Hydro alcoholic extract of *Annona squamosa* (L.) Leaves.

under 365 nm UV light, which may indicate the presence of water-soluble phytoconstituents such as tannins and glycosides.

**Table 2: Preliminary Phytochemical screening.**

| Chemical constituents | Hydroalcoholic extract |
|---|---|
| Carbohydrates | +++ |
| Proteins | ++ |
| Amino acids | Absent |
| Tannins | ++++ |
| Glycosides | +++ |
| Alkaloids | + |
| Flavonoids | +++ |
| Steroids | +++ |
| Saponins | +++ |

(+) = Low presence, (++) = Moderate presence, (+++) = High presence, (++++) = Abundant presence.

Ferric chloride treatment produced dark blackish fluorescence under UV light, suggesting the possible presence of phenolic compounds and tannins due to complex formation with ferric chloride. Acetic acid treatment showed orange fluorescence at 365 nm, which may be attributed to flavonoids and related phenolic compounds. Treatment with concentrated nitric acid produced orange brown colour in visible light and green fluorescence under UV light, indicating possible oxidation reactions of phytochemical constituents.

Ammonia treatment resulted in dark green and dark green/black fluorescence, which may be due to the presence of flavonoids and phenolic compounds. Ethanol and methanol treatments showed dark green fluorescence under visible light and UV light, indicating the presence of alcohol-soluble constituents such as flavonoids, glycosides, and tannins. Chloroform and petroleum ether treatments produced orange and dark brown fluorescence, suggesting the presence of non-polar constituents such as steroids and related compounds.

**Table 3: Fluorescence analysis of *Annona squamosa* Leaves Powder.**

| Treatment | Visible | 254 nm | 365 nm |
|---|---|---|---|
| Powder | Greenish brown | Dark green | Dark green/Black |
| Powder + Distilled water | Dark green | Dark green | Black |
| Powder + 5% $FeCl_3$ | Dark green | Dark green | Blackish |
| Powder + Acetic acid | Dark Brown | Dark green/Black | Orange |
| Powder + Con.$HNO_3$ | Orange brown | Green | Blackish |
| Powder + Ammonia | Dark green | Dark green | Dark green/Black |
| Powder + Ethanol | Dark green | Dark green | Dark green |
| Powder + Methanol | Green | Dark green/Black | Dark green |
| Powder + $CHCl_3$ | Dark green/Black | Dark green/Black | Orange |
| Powder + Pet. Ether | Dark green | Dark green | Dark brown |
| Powder + 10% aq. NaOH | Black | Dark green | Blackish |
| Powder + $H_2SO_4$ | Blackish | Black | Dark green |
| Powder + HCl | Dark green/Black | Black | Black |

**Figure 2:** Phytochemical screening of *Annona squamosa*.

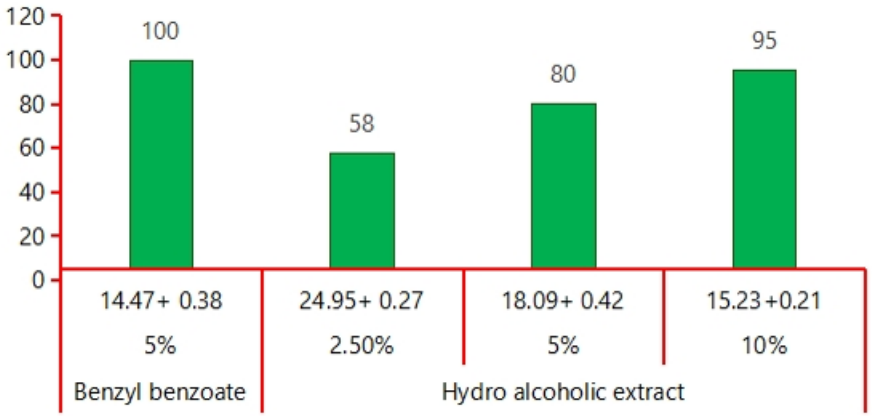


**Figure 3:** Anti-Lice Activity of Hydroalcoholic Leaf Extract of *Annona squamosa* Against Head Lice.

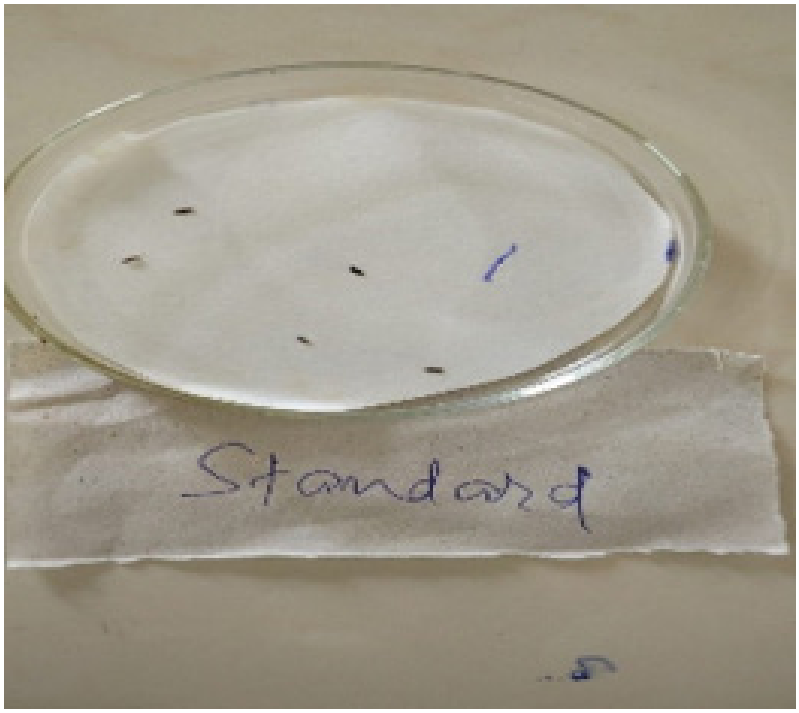


**Figure 4:** Anti-Lice Activity of Standard at 5% (Benzyl benzoate).

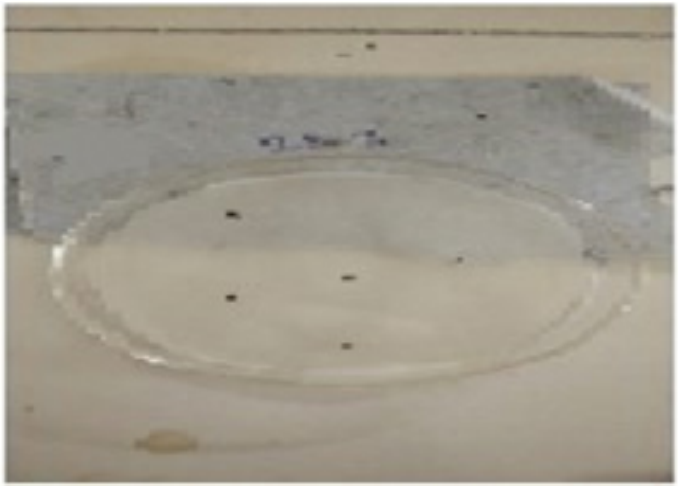

**Figure 5:** Anti-Lice Activity of Extract at 2.5%.

Treatment with aqueous sodium hydroxide produced black colour in visible light and dark green to blackish fluorescence under UV light, indicating the possible presence of phenolic and tannin compounds. Sulphuric acid treatment showed blackish colour in visible light, black fluorescence at 254 nm, and dark green fluorescence at 365 nm, suggesting a strong reaction with organic constituents. Hydrochloric acid treatment produced dark green/black fluorescence under UV light, indicating possible interaction of acidic conditions with certain phytoconstituents.

Overall, the fluorescence behavior of the leaves powder with different chemical reagents suggested the possible presence of important phytoconstituents such as tannins, flavonoids, glycosides, alkaloids, steroids, saponins, carbohydrates, and proteins. The fluorescence characteristics observed were found to be consistent with the preliminary phytochemical screening

**Table 4: Anti-Lice Activity of Hydroalcoholic Leaf Extract of *Annona squamosa* Against Head Lice.**

| Treatment | Concentration (%) | Mean time taken for death (min) | Average mortality rate (%) |
|---|---|---|---|
| Control (Hydroalcoholic solvent) | --- | --- | --- |
| Benzyl benzoate | 5% | 14.47± 0.38 | 100 |
| Hydro alcoholic extract | 2.5% | 24.95± 0.27 | 58 |
| | 5% | 18.09± 0.42 | 80 |
| | 10% | 15.23±0.21 | 95 |

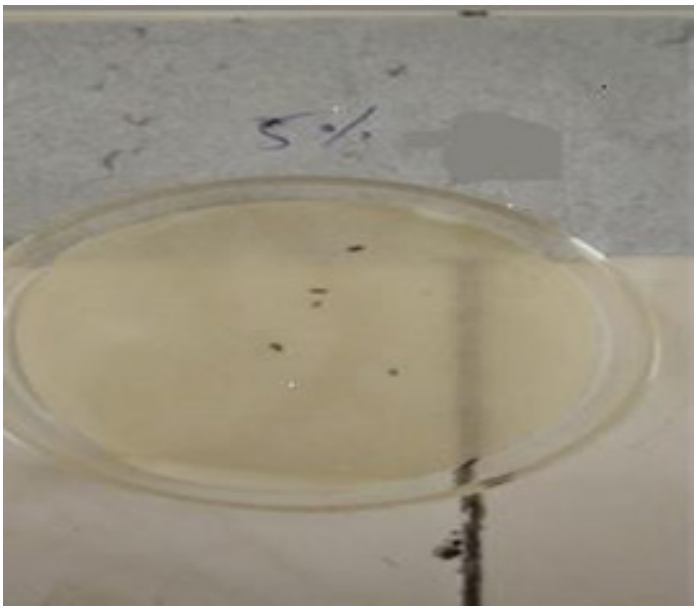


**Figure 6:** Anti-Lice Activity of Extract at 5%.

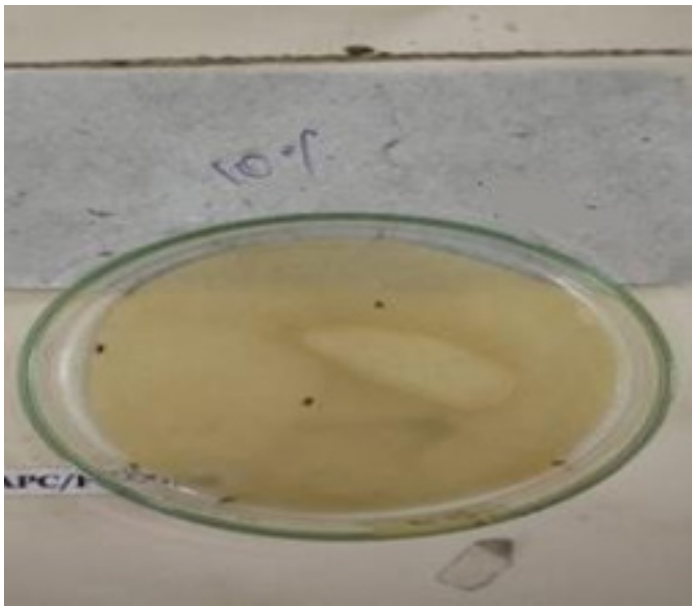


**Figure 7:** Anti-Lice Activity of Extract at 10%.

results of the hydroalcoholic extract. The results are shown in Table 3. Thus, fluorescence analysis can serve as a simple, rapid, and reliable pharmacognostic tool for the identification, authentication, standardization, and quality control of *Annona squamosa* leaves crude drug.

## Evaluation of Anti-Lice Activity of *ASHAE*

The hydroalcoholic extract of *Annona squamosa* leaves exhibited significant anti-lice activity in a concentration-dependent manner. The standard drug, 5% benzyl benzoate, produced 100% mortality within the shortest duration (14.47±0.38 min), indicating superior pediculicidal activity. Among the test samples, the 10% extract showed the highest activity with 95% mortality and a shorter mortality time compared to the lower concentrations. The 5% extract demonstrated considerable pediculicidal activity with 80% mortality, whereas the 2.5% extract showed moderate activity with 58% mortality.

No mortality was observed in the hydroalcoholic solvent-treated control group. The results indicate that the hydroalcoholic extract possesses significant pediculicidal potential, which may be attributed to the presence of phytoconstituents such as flavonoids, tannins, saponins, alkaloids, glycosides, and steroids identified during preliminary phytochemical screening. The anti-lice activity may be due to the synergistic effect of these bioactive constituents and the results shown in the Table 4, Graphically represented in Figure 3 and original images are shown in Figures 4-7.

## CONCLUSION

The present study was carried out to evaluate the phytochemical constituents and anti-lice activity of the hydroalcoholic extract of *Annona squamosa* leaves. The extraction process yielded a dark greenish-brown sticky extract with a satisfactory percentage yield, indicating the suitability of the hydroalcoholic solvent system for extraction of phytoconstituents from the plant material.

Preliminary phytochemical screening revealed the presence of several important bioactive constituents such as tannins, flavonoids, glycosides, steroids, saponins, carbohydrates, proteins, and alkaloids. Among these constituents, tannins were found in abundant quantity, while flavonoids, glycosides, steroids, and saponins were present in high amounts. These phytochemicals are known to possess various biological and pharmacological activities and may contribute to the observed pediculicidal activity of the extract.

The fluorescence analysis of the powdered leaves showed characteristic colour changes under visible light and UV light after treatment with different chemical reagents. These fluorescence characteristics can serve as important pharmacognostic parameters for identification, authentication, and standardization of *Annona squamosa* leaves as a crude drug.

The hydroalcoholic extract exhibited significant anti-lice activity against head lice in a concentration-dependent manner. Among the tested concentrations, the 10% extract showed the highest activity with 95% mortality and a shorter mean death time, which was found to be comparable with the standard drug benzyl benzoate. The 5% extract also demonstrated considerable pediculicidal activity, whereas the 2.5% extract showed moderate effectiveness. No mortality was observed in the control group treated with hydroalcoholic solvent, confirming that the observed activity was due to the plant extract itself.

The anti-lice activity observed in the study may be attributed to the synergistic action of phytoconstituents such as flavonoids, tannins, alkaloids, glycosides, steroids, and saponins present in the extract. These compounds may interfere with the physiological and metabolic activities of lice, resulting in paralysis and death.

Overall, the findings of the present study support the traditional use of *Annona squamosa* leaves in the treatment of lice infestation. The hydroalcoholic leaf extract demonstrated promising pediculicidal activity and may serve as a potential natural alternative to synthetic anti-lice agents. However, further studies involving isolation of active constituents, toxicity evaluation, formulation development, and clinical investigations are required to establish its safety, efficacy, and therapeutic application in the management of pediculosis.

## ACKNOWLEDGEMENT

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## ABBREVIATIONS

**ASHAE:** *Annona squamosa* Hydroalcoholic Extract; **UV:** Ultra Violet; **HCl:** Hydro Chloric Acid.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

The authors conceived and designed the study, performed the experiments, analysed and interpreted the data, prepared the manuscript, and approved the final version for publication.

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