

IN VITRO EVALUATION OF PEDICULOCIDAL POTENTIAL OF ANNONA MURICATA LEAVES

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ABSTRACT

Background: *Pediculosis capitis*, which is caused by *pediculus humanus capitis*, continues to be a common ectoparasitic infestation, especially among children of school age. The extended and indiscriminate use of synthetic pediculicides like permethrin and lindane has led to the emergence of resistance, decreased effectiveness, and adverse effects including scalp irritation and neurotoxicity. These drawbacks have heightened the quest for safer, more effective, and environmentally friendly alternatives. Medicinal plants that are abundant in bioactive phytochemicals have attracted significant interest as potential natural agents head lice. **Methods:** This study aimed to assess the *in vitro* activity of *Annona muricata* leaves extracts against *pediculus humanus Capitis* under controlled laboratory conditions. Plant materials were gathered, verified, shade-dried, and extracted with appropriate solvents (ethanol) following standard extraction protocols. Phytochemical screening was conducted to identify major secondary metabolites. Adult lice were collected and maintained under controlled conditions. The pediculicidal activity was evaluated using combined contact and fumigation bioassay methods at different concentrations such as 250, 500, and 750µg/mL. Mortality rates were recorded at predetermined intervals. **Results:** The findings indicated a time and concentration-dependent increase in the mortality rate of head lice. Higher extract concentrations produced a rapid pediculicidal effect and significantly shortened the survival time. Complete mortality of adult lice was noted at elevated concentrations within a shorter exposure duration compared to lower doses. Phytochemical analysis identified the presence of bioactive components such as alkaloids, flavonoids, terpenoids, phenolic compounds, and acetogenins, which may work together to produce the observed pediculicidal effects. **Conclusion:** The results from this investigation indicate that the *Annona muricata* leaves extract studied shows promising pediculicidal activity against *pediculus humanus capitis* and may represent a viable natural alternative to traditional synthetic pediculicides. Given its efficacy, plant- based origin, and anticipated safety, the extract could be further developed into an herbal formulation for pediculicidal use. However, further studies *in vivo* and clinical trials are necessary to confirm its safety, effectiveness, and formulation stability prior to therapeutic use.

KEYWORDS: *Pediculosis capitis*, *pediculus humanus capitis*, pediculicidal activity, medicinal plants, herbal pediculicide.

INTRODUCTION

Pediculus humanus capitis (head louse) is a blood sucking ectoparasite that is strictly reliant on humans for its lifecycle, classified under the order phthiraptera. It spends its entire life on the human scalp and feed multiple times daily, which makes dealing with infestations persistent and challenging. Head lice infestations continue to be one of the most prevalent parasitic issues globally, affecting people from all socioeconomic backgrounds, especially school aged

children. Transmission is primarily through close personal contact and the sharing of personal items like combs, hats, and bedding. Although head lice do not carry systemic diseases, they can cause severe itching, irritation of the scalp secondary bacterial infections, disturbances in sleep, social stigma and psychological stress, thereby presenting significant public health challenge.

The worldwide prevalence of pediculosis capitis shows considerable variation, with heightened rates among children aged 4 to 13 years. Factors such as overcrowding, lack of hygiene awareness, resistance to traditional pediculicides, and inadequate access to health care play role in its enduring nature. Chemical pediculicides, including permethrin, Malathion, lindane, pyrethrin, and ivermectin, have historically been employed for treatment. However their frequent use has resulted in growing resistance in louse population, diminished effectiveness, possible neurotoxicity, allergic responses, and environmental issues. These challenges emphasize the critical need for safer, more effective, and eco-friendly alternatives.

Products derived from plants have attracted attention as viable substitutes due to their biodegradability, low toxicity to mammals, and complex phytochemical makeup that lowers the chances of developing resistance. Essential oils and plant extracts possess bioactive elements like alkaloids, flavonoids, terpenoids, acetogenins, and phenolic compounds, many of which show insecticidal, ovicidal, and repellent characteristics. Their lipophilic property is facilitating penetration through the insect cuticle, result in neurotoxic and metabolic disturbance in ectoparasites.

Annona muricata L. (family: Annonaceae), commonly referred to as soursop or graviola, is a medicinal plant that is widely found in tropical areas. Phytochemical analysis has identified the presence of annonaceous acetogenins, alkaloids, flavonoids, tannins and essential oil –compound recognized for their powerful bioactivity against insects. Previous studies have shown that extract from *Annona muricata* exhibit larvicidal, insecticidal and repellent effect on various insect species.

Despite the increasing interest in plant based solution for lice, scientific evidence regarding effectiveness of *annona muricata* against the head lice is still limited. Consequently this current research was initiated to assess the pediculocidal property of *annona muricata* leaf extract under controlled laboratory condition and to evaluate it viability as a safe and effective alternative to traditional chemical treatments.

METHODS

1. Collection and extraction of plant materials

Fresh leaves of *Annona muricata* L. were gathered from the open fields in and around different parts of Kozhikode district during the harvest season and verified by a botanist. The leaves were rinsed, air dried in shade at room temperature, and ground into a powder using an electric grinder. The powdered material underwent solvent extraction using ethanol in a soxhlet apparatus. The extract was filtered and concentrated under reduced pressure with a rotary evaporator. The resultant dried extract was stored in airtight container.

2. Determination of biochemical constituents

Initial phytochemical screening were aimed to identify the presence of alkaloids, flavonoids, phenolic compounds, tannins, steroids, glycosides and acetogenins compounds responsible for their powerful bioactivity.

3. Preparation of test concentrations

The crude extract was dissolved in ethanol to create stock solutions. From these, various concentrations (2.5%, 5%, and 7.5%) were prepared for the pediculocidal bioassays. ethanol was used as the solvent control.

4. Test organisms

Live adults of *pediculus humanus capitis* were collected from infested school children using a fine-toothed lice comb. The collected lice were transferred to the laboratory in plastic containers along with strands of human hair. The samples were utilized within 2 hours of collection to ensure their viability.

5. Bioassay tests

For evaluating the pediculocidal activity was assessed using the combined contact filter paper and fumigation method. Whatman no.1 filter paper was treated with 1 mL of each extract concentration and allowed to air dry. Control papers are only treated with ethanol. The additional treated cotton was fixed to the inner surface of petridish lid, ensuring it didn't come into direct contact with lice, thereby enabling fumigant exposure. Groups of 10 active lice were placed in petridish and were covered with lids.

The petridishes were kept at room temperature. Mortality of lice was observed at 10, 20, 30, and 60 minutes respectively. Lice were deemed dead if there is no leg or gut movement observed, even after mild stimulation using forceps. Each experiment was replicated at least three times.

6. Data analysis

Mortality data were gathered at designated intervals for every concentration of the test sample. The count of decreased lice in each group was recorded, and the percentage mortality was calculated using the following equation.

$$\text{Percentage mortality (\%)} = \left(\frac{\text{number of dead lice}}{\text{total number of lice}} \right) \times 100$$

Results were expressed as mean $\pm$ standard derivation from triplicate experiments.

RESULT AND DISCUSSION

1. RESULT

1.1 Extract yield

Ethanolic extraction of *Annona muricata* leaves a dark green semisolid extract.

1.2 Preparation of leaf extract of *Annona muricata* & evaluation of percentage yield

Annona muricata's leaves are also known as soursop, a traditional remedy. The leaves were collected on

September 28-25 from several location in Kozhikode, Kerala. The powdered leaf material was extracted with ethanol using a soxhlet apparatus for 72 hours.

Table 1: Displays the percentage yield of *Annona muricata* leaf extract.

Name of plant	Plant part used	Method of extraction	Solvent used for extraction	Percentage yield(W/W)
<i>Annona muricata</i>	leaf	Soxhlet extraction	Ethanol	20%W/W

Table 1: percentage yield of *Annona muricata* leaf.

1.3 Phytochemical analysis

An ethanolic extract of *Annona muricata* leaf was subjected to preliminary phytochemical testing. The test aimed to identify alkaloids, tannins, flavonoids, saponins, phenolic compounds, steroids, glycosides, acetogenins. The ethanol extract contains several components such as alkaloids, tannins, flavonoids, steroids, and acetogenins. The summary of the preliminary phytochemical of *Annona muricata* leaf extract was shown in **table 2**.

Table 2: Phytochemical constituents in *Annona muricata* ethanolic leaf extract.

Si No	Name of constituents	Inference
1	Alkaloid	+
2	Flavonoids	+
3	Phenolic compounds	+
4	Tannins	+
5	Saponins	+
6	Steroids	+
7	Acetogenins	+
8	Glycosides	+

1.4 In vitro pediculocidal activity

Annona muricata leaf extracts effectiveness was assessed as part of the pediculocidal activity experiment. The extract showed notable pediculocidal activity that was concentration-dependent. At 750µg/mL. The highest mortality was seen, suggesting a high potential for pediculocidal effects. The leaves phytoconstituents, which include alkaloids, flavonoids, and acetogenins, may be responsible for the activity.

Table 3: concentration levels of *Annona muricata* leaf extract.

Treatment	Concentration(µg/mL)
T1	250
T2	500
T3	750

Annona muricata leaf extract concentration ranges from 250 to 750 µg/mL, as seen in **Table 3**. The necessary concentration was attained by preparing a stock solution of the extract with ethanol.

1.4.1 Combined Filter paper contact and Fumigation Bioassay



Fig 1: Head lice exposure into control treated petridish.



Fig 2: Head lice exposure into *Annona muricata* leaf extract.



Fig 3: combined filter paper & fumigation bioassay for different conc. of AMEE extract.



Fig 4: head lice exposure into permethrin treated petridish.

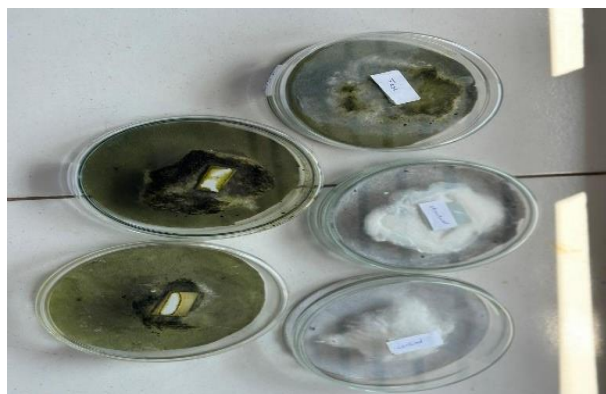


Fig 5: combined filter paper & fumigation bioassay control, test, (AMEE extract), permethrin.

Table 4: mortality rates of head lice after exposure to *Annona muricata* leaf extract 2.5%.

Time	Replicate 1	Replicate 2	Replicate 3	Mean
10 min	2	2	3	2
20 min	4	3	4	4
30 min	5	4	5	5
60 min	5	5	6	5

Table 5: Mortality rates of head lice after exposure to *Annona muricata* leaf extract 5%

Time	Replicate 1	Replicate 2	Replicate 3	Mean
10 min	3	3	4	3
20 min	5	5	6	5
30 min	6	5	6	6
60 min	6	6	7	6

Table 6: Mortality rates of head lice after exposure to *Annona muricata* leaf extract 7.5%.

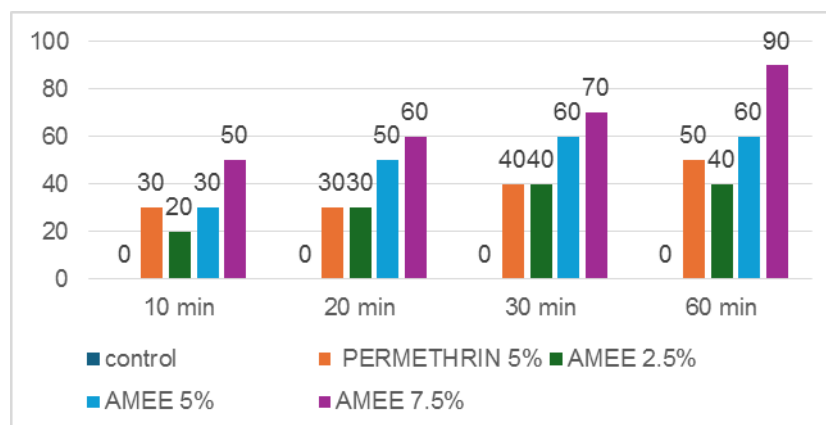
Time	Replicate 1	Replicate 2	Replicate 3	Mean
10 min	5	4	5	5
20 min	6	6	7	6
30 min	7	7	8	7
60 min	9	8	9	9

AMEE: *Annona muricata* ethanolic extracts

Percentage Mortality (%) = No. of dead lice ÷ Total no. of lice × 100

Table 7: Mortality rates of head lice after exposure to permethrin and *Annona muricata* leaf extract.

Treatment	n (Number of lice)	10 min		20 min		30 min		60 min	
		Dead	%	Dead	%	Dead	%	Dead	%
Control	10	0	0	0	0	0	0	0	0
Permethrin	10	3	30	3	30	4	40	4	40
AMEE 2.5%	10	2	20	4	40	5	50	5	50
AMEE 5%	10	3	30	5	50	6	60	6	60
AMEE 7.5%	10	5	50	6	60	7	70	9	90



Time dependent pediculocidal activity of *Annona muricata* ethanolic extract (AMEE) compared with permethrin.

AMEE pediculocidal activity increased over time, indicating a time –dependent effect. AMEE 7.5% had the strongest pediculocidal effectiveness among the tested concentrations at all-time points (10, 20, 30 and 60 minutes). AMEE 7.5% had the highest death rate (~90%) at 60 minutes, compared to lower doses of 2.5% and 5%. AMEE 5% demonstrated moderate but constant activity,

with higher mortality with longer exposure. AMEE 2.5% had the least degree of activity among the extract concentrations however it was still higher than the control. Permethrin (standard medication) had a quick pediculocidal effect but was comparable or slightly lower than AMEE 7.5% at later time points. The control group demonstrated no pediculocidal action, indicating that mortality was caused by the extract rather than solvent.

Overall, the data indicate the AMEE has concentration- and time-dependent pediculocidal action, with higher

concentrations demonstrating efficacy comparable to permethrin.

Table 8: Comparison Table: Mortality Distribution (%) at different time intervals.

Treatment	10 min	20 min	30 min	60 min
Control	0%	0%	0%	0%
Permethrin	23%	18%	19%	21%
AMEE 2.5%	15%	18%	19%	17%
AMEE 5%	23%	29%	29%	25%
AMEE 7.5%	32%	33%	35%	39%

The pie charts shows the mortality increases with concentration and over time.

- Among all treatments, AMEE 7.5% consistently made the biggest contribution to total mortality, followed by AMEE 5%.
- The findings support the idea that *Annona muricata* ethanol extract has potent pediculocidal activity, particularly at higher concentrations.

2. DISCUSSION

Pediculosis capitis, caused by *Pediculus humanus capitis*, remains a common ectoparasitic infestation. The extensive use of synthetic pediculicides has led to resistance and adverse effects such as scalp irritation, allergic reactions, reducing their long-term effectiveness and highlighting the need for safer plant-based alternatives.

The contact toxicity of *Annona muricata* leaf extract against *pediculus humanus capitis* showed a clear time- and concentration-dependent effect. Mortality increases progressively with exposure time (10-60 minutes) and higher extract concentrations.

Significant differences in mortality means were observed across exposure times (10, 20, 30 and 60 min) indicating values. At that time significantly influenced pediculocidal activity. Similarly, extract concentration had a significant effect on head lice mortality, with higher concentrations producing faster knockdown and higher mortality rate.

The findings are consistent with reports on other medicinal plants such as *Azadiracta indica*, *Melaleuca alternifolia*, and *Eucalyptus globulus*, which also exhibit dose-dependent pediculocidal activity. The comparatively lower effectiveness of permethrin observed in this study may be attributed to the development of resistance.

The extract exhibited both contact and fumigant modes of action, indicating multiple mechanisms of activity. This effect may be due to the presence of bioactive phytochemicals such as acetogenins, alkaloids, flavonoids, and terpenoids. Acetogenins are known to disrupt mitochondrial function, leading to energy depletion and death of the parasite.

Overall efficacy

The findings indicate that *Annona muricata* leaf extract has considerable pediculocidal effect. The activity is significantly affected by both concentration levels and duration of exposure, highlighting its potential as a plant-based alternative to chemical pediculicides.

CONCLUSION

The current study found that the ethanolic leaf extract of *Annona muricata* has high in-vitro pediculocidal activity against *Pediculus humanus capitis*, indicating that it may play a role in pediculosis therapy. When the extract was tested utilizing a combination contact and fumigation bioassay, there was a definite dose- dependent increase in lice mortality. Higher quantities of the extract resulted in increased pediculocidal efficacy, indicating its promise as a natural medicinal agent. There was no significant mortality in the ethanol control group, indicating that the pediculocidal action was simply due to the plant extract. Permethrin, the conventional medication, caused rapid and complete mortality, supporting the experimental model.

The observation action of *Annona muricata* leaves could be due to the presence of bioactive phytochemicals with known insecticidal activities. Overall, these data indicate that, with additional investigation and formulation, *Annona muricata* leaf extract could be a promising herbal alternative to standard synthetic pediculicides. Furthermore, the extract demonstrated significant efficacy in both contact and fumigation assay, indicating its potential. Further research should focus on the isolation and characterization of active substances, as well as safety evaluation and formulation development, in order to develop an effective and safe herbal treatment for pediculosis.

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