

Effect of aqueous extracts of *Pithecellobium dulce* (Roxb.) Benth against *Pediculus humanus capitis* De Geer, 1778 (Procodea, Pediculidae)

Susmita Gudulkar*, Himanshu Dawda, Usha Mukundan and Somnath Kangralkar

Department of Botany, Plant Biotechnology Laboratory, Ramniranjan Jhunjhunwala College, Ghatkopar (West) 400086, Mumbai, India.

Email: sgudulkar2@gmail.com; drhimanshudawda@gmail.com; usha.rjc@gmail.com

ABSTRACT: The study explores a safe and natural approach to louse management through the use of extracts derived from *Pithecellobium dulce* (Roxb.) Benth., commonly known as Manila tamarind. The efficacy of the aqueous extracts was assessed based on their ability to kill lice and their eggs, employing the filter paper diffusion method. The extracts from leaf, fruit, bark and stem of the plant revealed a mean mortality of 66.7, 40.0, 26.7 and 53.3 per cent respectively against nymphs and adults. The extracts were effective on nits, on the tenth day. The average emergence of nymphs, from the nits treated with leaf, fruit, bark and stem extracts were 06.7, 73.3, 66.7 and 20.0 per cent respectively. The study revealed that the aqueous extract of the leaf of *P. dulce* exhibited remarkable antilice and ovicidal properties.

© 2025 Association for Advancement of Entomology

KEY WORDS: Manila tamarind, pediculicide, saponin, anti-lice

INTRODUCTION

The relationship between parasites and their hosts has fascinated scientists for centuries, with some of the most complex interactions occurring between ectoparasites and mammals. Among these, lice classified under the order Phthiraptera and suborder Anoplura, stand out due to their specialized adaptations. They are also known as sucking lice, and these ectoparasites sustain themselves by feeding on the blood of placental mammals, including humans, for survival (Durden and Musser, 1994). Having identified over 530 species, lice are highly host-specific, often evolving to thrive on particular mammalian hosts (Durden and Musser, 1994; Amanzougaghene *et al.*, 2020). Lice

infestations lead to severe itching. This irritation can result in symptoms such as pruritus and eczematous changes (Mumcuoglu *et al.*, 2021; Muršič *et al.*, 2018; Guenther, 2015). Individuals with head lice infestations commonly report itching due to the irritative effects of lice saliva (Mumcuoglu *et al.*, 2021).

Lice infestation is not only a health problem, but also a social problem, particularly affecting children, who may experience anxiety, depression, and social isolation due to the stigma associated with these parasites (Babazadeh *et al.*, 2023; Banafshi and Khatony, 2024). Lice infestation occurs primarily through close physical contact or sharing personal items such as hats, combs, towels, etc. (Firoozfar

* Author for correspondence

et al., 2019). Although effective, these chemical treatments often have drawbacks, including side effects such as skin irritation and the development of resistance among lice populations (Ghavami *et al.*, 2023). Additionally, it may not effectively kill lice eggs, necessitating repeated treatments (Wismer, 2016). Synthetic pediculicides typically work by killing lice by disrupting the functioning of their nervous system (Nolt *et al.*, 2022), but growing concerns about chemical resistance necessitate research into safer alternative methods to control lice infestations. Bioinsecticides are highly effective, safe, and ecologically acceptable. These properties make bioinsecticides a promising alternative for managing head lice infestations, as they reduce the risk of environmental pollution and adverse health effects associated with synthetic chemicals (Anila and Hashim, 2023). *Pithecellobium dulce* (Roxb.) Benth, commonly known as “Manila tamarind,” is rich in secondary metabolites such as saponins, flavonoids tannins, phenols and terpenoids (Srinivas *et al.*, 2018; Murugesan *et al.*, 2019). These secondary metabolites work together to effectively combat lice by disrupting their cellular structure and metabolic processes (Katekhaye and Kale, 2012; Kumar *et al.*, 2013; Kondabolu *et al.*, 2023). The study explores a safe and natural approach to lice management through the use of extracts derived from *P. dulce*. The efficacy of the extracts was assessed based on their ability to kill lice and their eggs, employing the filter paper diffusion method.

MATERIALS AND METHODS

Collection and authentication of the plant

material: The leaves, fruits, bark, and stems of *P. dulce* were collected from Ghatkopar, Mumbai, India. These plant materials were identified and taxonomically authenticated by the Blatter Herbarium at St. Xavier’s College in Mumbai, Maharashtra, India. The collected specimen corresponded to specimen number JP-3229, originally cataloged by J. Pallithanam in the Blatter Herbarium collection (Kumaran, 2018).

Preparation of aqueous plant extract: The various parts were collected, shade dried, and finely powdered using a laboratory grinder. The powdered

material was subsequently sieved through a 180µm mesh to ensure uniform particle size. To prepare a five per cent aqueous decoction, 5g of plant powder was refluxed in 100 mL of distilled water at 90°C for one hour (Ennaifer *et al.*, 2018; Rudraswamy *et al.*, 2021). Following the refluxing process, the extract was allowed to cool at ambient temperature. The cooled decoction was subsequently filtered by using Whatman filter paper no. 1 to remove any residue. The filtrate was then dried in a hot air oven at 70-75°C until completely desiccated. The reconstituted extract obtained from this process was utilized for further analysis in the study.

Specimens of *Pediculus humanus capitis*, comprising of adults, nymphs and nits, were collected from children aged 8 to 12 years and transferred into sterile plastic containers. The container lids were loosely secured with parafilm to permit ventilation, and samples were promptly taken to the research laboratory for the study. Testing for lice and ovicidal effects *in vitro* began within one hour of collection. Using a stereo zoom microscope (MICRON OPTIK-PRIME), preliminary examination was conducted to identify and separate adult lice and nymphs for further study (Samuel *et al.*, 2009; Vijayalakshmi *et al.*, 2010; Sherwani *et al.*, 2013).

Antilice activity: To study the pediculocidal activity of aqueous extracts obtained from different parts, a filter paper diffusion methodology was employed (Sherwani *et al.*, 2013). The plant extracts were dissolved in distilled water to prepare two concentrations (2 and 5%). Preliminary results indicated no significant difference in efficacy between two concentrations, so only the data for the two per cent concentration are presented here. Test subjects, consisting of adult lice and nymphs at a ratio of 3.6/1.4, were classified into five groups with five lice per group. Each group was carefully positioned on Whatman No. 1 filter paper at the base of a petri dish (55mm diameter, 10mm height), which remained uncovered. Consecutively, 0.5mL of the respective aqueous extract was applied directly onto the lice in each Petri dish, allowing the extract to spread evenly in a thin layer (Samuel *et al.*, 2009; Sherwani *et al.*, 2013; Candy *et al.*,

2018). The petri dishes were subsequently moved to a dark and humid chamber maintained at $26 \pm 0.5^\circ\text{C}$ with 70 ± 1 per cent relative humidity. The study was conducted by forming groups (Group I-V). Group I, treated with 0.5mL of distilled water, served as the control, while Groups II through V received the 0.5mL of plant extracts. Observations were carried out at one-hour intervals using a stereo zoom microscope. After five hours, the petri dishes were re-examined under the stereo zoom microscope, and the lice showing no movement were confirmed as dead (Yingklang *et al.*, 2022). All the experiments were performed in triplicate and repeated twice.

Ovicidal Activity: Head louse eggs were examined using a stereo zoom microscope and categorized into early stages, characterized by the presence of a yolk cell, or late stages, identified by the appearance of eye spots. This classification was performed prior to the conduct of the tests. The ovicidal activity was assessed by positioning five brown, oval-shaped eggs with intact operculum on Whatman filter paper no. 1, placed at the base of each petri dish (55mm diameter, 10mm height). A volume of 0.5mL of each test solution and control was carefully applied to the eggs. The dishes were subsequently incubated in a dark chamber maintained at $26 \pm 0.5^\circ\text{C}$ for 14 days. To ensure adequate moisture, 0.1mL of distilled water was added every 48 hours. The hatching process was monitored microscopically, and the percentage of emergence was observed. Each experimental treatment was performed in triplicate and repeated twice to ensure reliability (Samuel *et al.*, 2009; Yingklang *et al.*, 2022).

One-way analysis of variance (ANOVA) was performed between treatment and control groups to determine the significance difference in the mortality/nymph emergence. Tukey's test was performed to determine the difference between the mean percentage mortality/ nymph emergence. Statistical significance was established at the $p < 0.05$.

RESULTS AND DISCUSSION

The aqueous extracts of different parts of *P. dulce*

were effective against nymph and adults of *P. humanus capitis*. The average mortality of adults was 66.7 per cent with the leaf extract, followed by stem extract by 53.3 per cent, fruit extract by 40.0 per cent and bark extracts by 26.7 per cent. Leaf extracts showed significant mortality at all-time points followed by stem extracts (Fig. 4) compared to control groups which are treated with distilled water (showed no mortality during 5 hours assessment). The experimental images showing mortality of lice after treatment with aqueous extracts from different parts of *P. dulce* are presented as leaf - Fig. 1C, fruit - Fig. 1D, bark - Fig. 1E, and stem - Fig. 1F.

Evaluation of aqueous extracts of different parts of *P. dulce* against nits of *P. humanus capitis*, revealed their effectiveness in preventing the emergence/ hatchability. The average emergence of nymphs on day 10 was very low upon treatment with leaf extract (6.7%) and significantly superior compared to control group which has shown an emergence of 93 per cent, indicating its potent ovicidal efficacy. Others extracts from stem, fruit and bark have shown very milder effects on emergence level by 20, 73.3 and 66.7 respectively (Table 1). There was normal developmental progression of hatched egg in control group in distilled water (Figs. 2A – F), while the developmental progression of egg treated with the different aqueous extracts of *P. dulce* were significant (Fig. 3A-D). The results indicated that

Table 1. Percentage of Nymph emergence at different intervals (0-10 days) after application of aqueous extract of *P. dulce* leaf, bark, fruit, stem and water

Percentage of nymph emergence			
Part	Day 0-5	Day 6-10	Total at Day 10
Fruit	33.33 $\pm$ 11.54	40.00 $\pm$ 20.00	73.33 $\pm$ 31.54
Leaf	6.66** $\pm$ 11.54	0.00** $\pm$ 0.00	6.66** $\pm$ 11.54
Bark	26.66 $\pm$ 11.54	40.00 $\pm$ 0.00	66.66 $\pm$ 11.54
Stem	0.00 $\pm$ 0.00	20.00 $\pm$ 20.00	20.00 $\pm$ 20.00
Water	20.00 $\pm$ 0.00	73.33 $\pm$ 11.54	93.33 $\pm$ 11.54

Values in column followed by ** are significant at p value < 0.05 as per Turkey's test; [N=5 (Experiment repeated=3)]

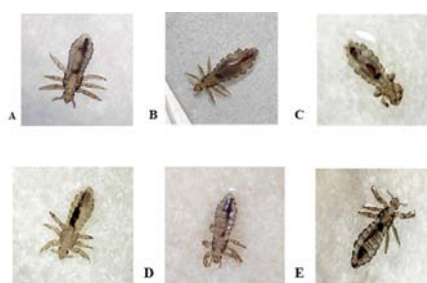


Fig. 1 A. Nymph and B. Adult stages of human lice showing no mortality in the control group. Fig. 1C-F. Antilice activity resulting in lice mortality in - C. leaf extract, D. fruit extract, E. bark extract and F. stem extract

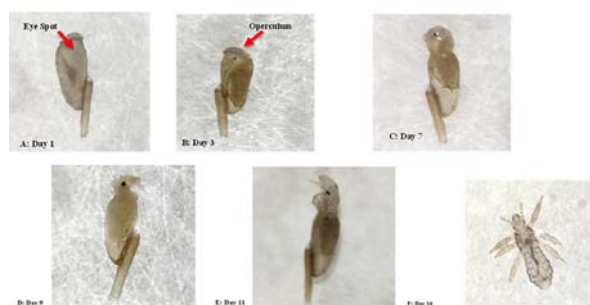


Fig. 2 Developmental progression of hatched eggs (A to F) in control groups (Distilled Water)

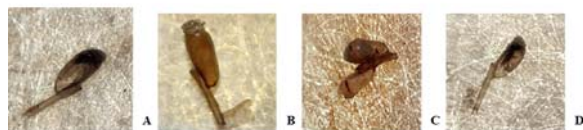


Fig. 3 Ovidical activity showing unhatched eggs in the treatments - A. leaf extract, B. fruit extract, C. bark extract and D. stem extract

the aqueous extracts derived from different parts of *P. dulce* exhibited notable activities against lice and their eggs. This bioactivity is likely due to a variety of secondary metabolites present, such as saponins, phenols, flavonoids, terpenoids, and tannins (Katekhaye and Kale, 2012; Kumar *et al.*, 2013; Kondabolu *et al.*, 2023) which may work together to effectively act as antilice agents. Saponins are heterosides, sourced from plants and consist of one or more sugar units, demonstrate considerable potential as pesticides. When applied as an active substance against hatched lice, saponins affect the cell protoplasm, which is a liquid crystalline framework. This interaction results in the solidification of the protoplasm, thereby creating holes in the cell membrane. As a result, the cell fluids escape, ultimately leading to death of the lice (Diaz *et al.*, 2015). Furthermore, tannins and phenolic compounds could serve a vital role in the effectiveness of the extract. Tannins are capable of penetrating the outer membrane of lice,

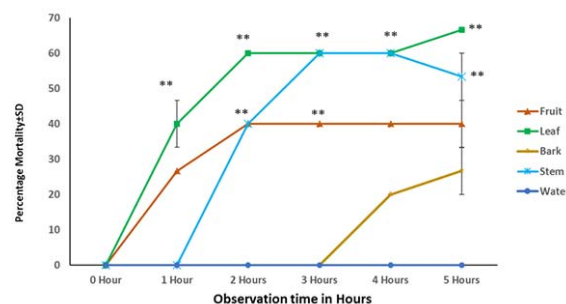


Fig. 4 Percent of head lice mortality at different time intervals (0-5 Hour) after application of aqueous extract of *P. dulce* leaf, bark, fruit stem and water. **significant at $p < 0.05$ as per Turkey's test

compromising cellular structure and obstructing nutrient uptake, which can lead to starvation and death. Their phenolic compound might cause tissue shrinkage and block proteins on the lice's surface, disrupting essential functions. Additionally, phenolic compounds may enhance these impacts by interfering with metabolic processes, reducing nutrient absorption, and inducing overall dysfunction (Samiasih *et al.*, 2023). Together, these compounds could target both the structural and metabolic systems of lice, enhancing the extract's anti-lice efficacy. Unlike synthetic pediculicides like permethrin, which can leave residues contributing to resistance (Samuel *et al.*, 2009), natural extracts from *P. dulce* offer a safer, sustainable solution with diverse modes of action that minimize resistance risk.

Several studies have demonstrated the effectiveness of plant-based extracts such as *Melia azedarach* L. (Carpinella *et al.*, 2007), *Callistemon viminalis* essential oil (Yingklang *et al.*, 2022), and *Pongamia pinnata* petroleum ether extract (Samuel *et al.*, 2009) in treating lice infestations through significant, dose and concentration-dependent anti-lice and ovicidal properties. The results of the present study emphasize the effectiveness of extracts in controlling lice outbreaks and reducing egg emergence. Among the various extracts evaluated, the leaf extract exhibited the highest efficacy in eliminating lice and inhibiting egg hatching. The stem extract also demonstrated notable effectiveness against lice and in preventing

eggs from hatching, while the fruit and bark extracts were less potent but still surpassed the control group that received distilled water. These findings indicate that *P. dulce* leaf extract, presents a strong natural alternative for synthetic treatments.

ACKNOWLEDGEMENT

The authors extend their gratitude to the Institute and the management of Ramniranjan Jhunjhunwala College, Mumbai, for their generous provision of lab facilities.

REFERENCES

- Amanzougaghene N., Fenollar F., Raoult D. and Mediannikov O. (2020) Where are we with human lice? A review of the current state of knowledge. *Frontiers in Cellular and Infection Microbiology* 9: 474.
- Anila L. and Hashim M.S. (2023) Evaluation on the insecticidal action of polyphenolic compound from *Streblus asper* (Lour.) on the red cotton bug, *Dysdercus cingulatus* (Fab.) (Hemiptera, Pyrrhocoridae). *ENTOMON* 48(4): 531–536.
- Babazadeh T., Chollou K. M., Abedi Nerbin S., Abedi Nerbin S., Shahnavaiz Yoshanluie F. and Ranjbaran S. (2023) Head lice infestation and the role of some cognitive behavioral factors in its spread and prevention among adolescent girls: A cross sectional study in Northwest Iran. *Health Science Reports* 6(11): e1679.
- Banafshi Z. and Khatony A. (2024) Exploring the lived experiences of adolescent girls affected by head lice infestation: a qualitative descriptive study. *BMC Infectious Diseases* 24(1): 825.
- Candy K., Nicolas P., Andriantsoanirina V., Izri A. and Durand R. (2018) *In vitro* efficacy of five essential oils against *Pediculus humanus capitis*. *Parasitology research* 117 (2): 603–609.
- Carpinella M. C., Miranda M., Almirón W. R., Ferrayoli C. G., Almeida F. L. and Palacios S. M. (2007) *In vitro* pediculicidal and ovicidal activity of an extract and oil from fruits of *Melia azedarach* L. *Journal of the American Academy of Dermatology* 56(2): 250–256.
- Diaz Danica A. (2015) Effectivity of the shampoo formulated from saponin twig extract of the *Artocarpus altilis* (rimas) against *Pediculus humanus capitis*. Our Lady of Fatima University, Quezon. Thesis. 37 pp.
- Durden L.A. and Musser G.G. (1994) The sucking lice (Insecta, Anoplura) of the world: a taxonomic checklist with records of mammalian hosts and geographical distributions. *Bulletin of the American Museum of Natural History* no. 218. 90pp.
- Ennaifer M., Bouzaïene T., Chouaibi M. and Hamdi M. (2018) *Pelargonium graveolens* aqueous decoction: A new water soluble polysaccharide and antioxidant rich extract. *BioMed Research International* 2018(1): 2691513.
- Firoozfar F., Moosa-Kazemi S. H., Bahrami A., Yusuf M. A., Saghaipour A., Armoon Z., Rajabzadeh R. and Hosseini S. H. (2019) Head lice infestation (*Pediculus humanus capitis*) prevalence and its associated factors, among the Kormanj tribes in North Khorasan province. *Shiraz E-Medical Journal* 20(4). e80292. doi: 10.5812/semj.80292
- Ghavami M.B., Panahi S., Nabati S.M., Ghanbari M. and Taghiloo B. (2023) A comprehensive survey of permethrin resistance in human head louse populations from northwest Iran: *ex vivo* and molecular monitoring of knockdown resistance alleles. *Parasites & Vectors* 16(1): 57.
- Guenther L.C. (2015) Pediculosis and phthiasis (lice infestation). Diunduh dari <https://emedicine.medscape.com/article/225013-clinical>.
- Izzah I.N.L., Suci S.E., Dewi Y. and Yuniarsih N. (2022) The potential of natural ingredients as anti pediculosis capitis: A narrative review. *Archives of The Medicine and Case Reports* 3(4): 300–303.
- Katekhay S.D. and Kale M.S. (2012) Antioxidant and free radical scavenging activity of *Pithecellobium dulce* (Roxb.) Benth wood bark and leaves. *Free radicals and antioxidants* 2(3): 47–57.
- Kondabolu U.L., Babitha B., Kalagatur N.K., Nagaraj A. and Velumani S. (2023) Phytochemical analysis in *Pithecellobium dulce* fruit peel extract. *Current Trends in Biotechnology and Pharmacy* 17(3): 1052–1059.
- Kulkarni K.V. and Jamakhandi V.R. (2018) Medicinal uses of *Pithecellobium dulce* and its health benefits. *Journal of Pharmacognosy and Phytochemistry* 7(2): 700–704.
- Kumar M., Nehra K. and Duhan J.S. (2013) Phytochemical analysis and antimicrobial efficacy of leaf extracts of *Pithecellobium dulce*. *Asian Journal of Pharmaceutical and Clinical Research* 6(1): 70–76.

- Kumaran N.S. (2018) Evaluation of *in vivo* antidiabetic and antioxidant activity of *Achyranthes aspera* Linn. seeds by streptozotocin induced diabetic rats. *International Journal of Green Pharmacy* 12(1): 29–37. doi:10.22377/ijgp.v12i01.1520.
- Mumcuoglu K.Y., Pollack R.J., Reed D.L., Barker S.C., Gordon S., Toloza A.C., Picollo M.I., Ozkan A.T., Chosidow O., Habedank B., Ibarra J., Meinking T.L. and Vander Stichele R.H. (2021) International recommendations for an effective control of head louse infestations. *International Journal of Dermatology* 60(3): 272–280.
- Muršić I., Kuric I., Ragu• M., Kovačević T. and Muršić D. (2018) Treatment of scabies and pediculosis in health education publications and folk medicine of Eastern Croatia-Slavonija, Baranja and Western Srijem county. *Acta Dermatovenerologica Croatica* 26(1): 33–33.
- Murugesan S., Lakshmanan D.K., Arumugam V. and Alexander R.A. (2019) Nutritional and therapeutic benefits of medicinal plant *Pithecellobium dulce* (Fabaceae): A review. *Journal of Applied Pharmaceutical Science* 9(7): 130–139.
- Nolt D., Moore S., Yan A.C. and Melnick L. (2022) Committee on infectious diseases, committee on practice and ambulatory medicine, section on dermatology. Head lice. *Pediatrics* 150(4): e2022059282.
- Rudraswamy S., Godhi B., Shankar H.P.J., Kenganora M. and Sumana M.N. (2021) Detailed understanding of different extraction methods for the research on medicinal plants. *Indian Journal of Oral Health and Research* 7(1): 14–20.
- Samiasih A., Dianingsih A., Ferdisa R.J., Wati F., Hartiti T., Ernawati A.Y. and Yanto A. (2023) The effectiveness of garlic, black turmeric, and red betel vine solutions to maintain scalp hygiene (pediculicide efficacy test toward head lice). *South East Asia Nursing Research* 2(4): 131–138.
- Samuel A.J.S.J., Radhamani S., Gopinath R., Kalusalingam A., Vimala A.G.K.A. and Husain A. (2009) *In vitro* screening of anti-lice activity of *Pongamia pinnata* leaves. *The Korean Journal of Parasitology* 47(4): 377.
- Sherwani S.K., Ahmad H., Aijaz M., Kausar R., Sarwar M.I., Nangyal H. and Kazmi S. U. (2013) Anti-head lice activity of *Camellia sinensis* (Green tea) aqueous decoction, infusion and microwave assisted crude extracts. *Journal of Pharmacognosy and Phytochemistry* 2(4): 189–192.
- Srinivas G., Geeta H.P., Shashikumar J.N. and Champawat A. (2018) A review on *Pithecellobium dulce*: A potential medicinal tree. *International Journal of Chemical Studies* 6(2): 540–544.
- Vijayalakshmi M., Periyayagam K. and Prabu S.L. (2010) *In vitro* antilice activity of *Dichrostachys cinerea* (L.) Wight & Arn. *International Journal of PharmTech Research* 2(4): 2210–2213.
- Wisner T. (2016) Feline Toxins: Recognition, Diagnosis, Treatment. In: *August's Consultations in Feline Internal Medicine*, Volume 7. WB Saunders. pp791–798.
- Yingklang M., Priprem A., Nualkaew N., Sungthong B., Pinlaor P. and Pinlaor S. (2022) Biochemical constituents and insecticidal activities of *Callistemon viminalis* essential oil against adults and eggs of *Pediculus humanus capitis* (Phthiraptera: Pediculidae). *Phytomedicine Plus* 2(1): 10015.

(Received May 12, 2025; revised ms accepted August 02, 2025; published September 30, 2025)