

Evaluating the antimicrobial potential of selected plant essential oils against major pathogens of *Antheraea assamensis*

Dhirunabh Swargiary¹, Anjumoni Ojha², Anurupa Goswami^{3*}, Selima Nasrin¹, Manoj Barthakur²

¹ Department of Botany, B. Borooah College, Guwahati, Assam, India

² DBT-NER Advanced Level Institutional Biotech Hub, B. Borooah College, Guwahati, Assam, India

³ Department of Zoology, B. Borooah College, Guwahati, Assam, India

Corresponding Author: Dr. Anurupa Goswami

Abstract

In Assam, the sustainable production of muga silk is hampered by bacterial and fungal disease, resulting in considerable loss of the crops and negative effects on the economy. This study evaluated the ability of essential oils from three medicinal plants such as *Cinnamomum verum*, *Litsea salicifolia* and *Andrographis paniculata* to inhibit major diseases that afflict *Antheraea assamensis* and to evaluate the antimicrobial activity (antimicrobial peptides, AMP) of their silkworm haemolymph. Essential oils were tested for antimicrobial activity using the well diffusion method for *Bacillus thuringiensis*, *Serratia marcescens* and *Beauveria bassiana*. Antimicrobial effects were found to differ, with the most potent antifungal activity of essential oil obtained from *C. verum* against *B. bassiana*, whilst *L. salicifolia* provided the strongest antibacterial activity against *B. thuringiensis*. Gas chromatography mass spectrometric (GC-MS) study showed that most of the major constituents contributing to the antimicrobial inhibition were found to be bioactive metabolites, including: phenylacetaldehyde, coumarin, benzyl benzoate, cinnamic acid, longifolene, and dodecane. Consequently, these findings indicate that the bioactive metabolites found in pathogen-infected silkworms may induce humoral immune responses as silkworm haemolymph extracts had a high level of antimicrobial activity; thus, presenting an opportunity to integrate plant essential oil with silkworm factors into an eco-friendly alternative to traditional chemical applications for the control of disease in silkworms, ultimately leading to enhanced sustainable muga silk production.

Keywords: *Antheraea assamensis*, essential oils, antimicrobial peptides, sericulture disease management, GC-MS

Introduction

Silkworms have been an important part in the growth of human societies for many centuries acting as a basis for sericulture and textile industry as well as for the building of our economy. Assam, the North Eastern state of India is an important producer of silkworms and plays an important role in the sericulture industry, particularly with its production of the endemic silkworm *Antheraea assamensis* (Devi *et al.*, 2021) [10]. Like many traditional crafts in Assam, sericulture is not just an industry but also a part of the culture of Northeastern India. One of the most well-known types of Assam silk is Muga silk which has been given a geographical indication (GI) tag to signify the uniqueness of the product (Goswami *et al.*, 2016) [15]. Sericulture provides significant contributions to economies based on agriculture globally, with millions of jobs being created in developing nations working in the silk industry (Dash *et al.*, 2023) [9]. The fibroin and sericin proteins produced by silkworms have been studied widely for their biocompatibility and have been used to create many products in the health care, cosmetics industries, and many biological fields (Luo *et al.*, 2025) [28].

Silkworm rearing generally has many challenges including the potential for them to contract one or more diseases from a variety of sources, such as Flacherie, Grasserie, Muscardine, and Pebrine, because of biological, chemical, physical, nutritional and environmental factors. All these diseases lead to poor-quality raw silk and crop loss due to reduced production (Tikader *et al.* 2013) [41]. Among these diseases, flacherie and bacterial septicaemia account for the most serious types of problems and are the primary causes

of increased cocoon crop losses (Tayal & Chauhan 2017, Jigyasu *et al.*, 2023 and Ishii *et al.*, 2014) [21, 24, 40]. Flacherie is caused by both bacterial and viral pathogens. Among the main bacterial agents causing Flacherie are *Bacillus thuringiensis*, *Streptococcus sp.*, and *Staphylococcus sp.* However, *Serratia marcescens* causes bacterial septicaemia leading to high mortality rates in silkworms (Zhang *et al.* 2020) [42]. The major fungal pathogen affecting silkworms is *Beauveria bassiana*, which typically infects silkworms by invading their cuticle and causing white muscardine disease and subsequently leading to significant losses in cocoon production (Huo *et al.* 2014). Chemical antimicrobial agents were previously the primary means of controlling diseases in silkworms; however, due to growing concerns about the development of pathogen resistance to chemical agents, environmental toxicity, and bioaccumulation of chemical agents, there is now an urgent need to find sustainable, environmentally friendly alternatives such as plant-derived antimicrobial agents.

Plant essential oils are receiving significant interest as they possess a variety of antimicrobial properties, have low levels of environmental persistence, and have minimal toxicity (Bakkali *et al.*, 2008) [4]. The potential use of plant essential oils and extracts in disease control has resulted from their broad-spectrum antimicrobial and immunomodulatory properties (Isman *et al.* 2020) [22]. These bioactive compounds such as phenols, terpenoids, flavonoids, and alkaloids are very effective against various types of pathogens, and do not have any harmful effects to the environment (Bakkali *et al.*, 2008) [4]. Among the other plants, some locally available plants such as *Litsea*

salicifolia, *Cinnamomum verum*, *Andrographis paniculata*, etc. have been shown to possess bioactive components that have been shown to have antibacterial and antifungal activities (Puppala *et al.*, 2023 [34]; Seyed Ahmadi *et al.*, 2019; Chao and Lin, 2010) [7, 38]. *Litsea salicifolia* is a plant known for its fragrant leaves and high concentration of essential oils, both of which have been shown to have antimicrobial properties (Kalita *et al.*, 2016) [19]. *C. verum* is another aromatic species with a relatively high concentration of essential oil containing cinnamaldehyde that has been extensively studied for its antimicrobial and antioxidant properties (Al-Garadi *et al.*, 2023) [3]. Chemical metabolites isolated from these plants have demonstrated several antimicrobial properties when tested. For example: Cinnamaldehyde which is one of the major components in *C. verum* has been shown to (Guo *et al.*, 2024) [17]. *Andrographis paniculata* is commonly referred to as "King of Bitters." Based on its chemical composition, *Andrographis paniculata* is rich in antioxidant, antibacterial, antifungal, and antiviral properties (Mishra *et al.*, 2007) [29]. The present study aims to control the fungal and bacterial disease-causing pathogens by using the essential oil and bioactive metabolites extracted from some locally available medicinal plants. The controlling efficacy of antimicrobial peptide (amps) derived from haemolymph of disease infected silkworm will also be used to control the pathogens. However, integration of phytotherapy and immunomodulatory strategies, is the prime objective for development of eco-friendly disease management practices to ensuring healthier silkworm populations and improved silk yield.

Materials and methods

1. Insect rearing

The study was conducted using healthy and matured 5th instar 3rd day Muga Silkworms. The larvae were collected from the silkworm rearing farm of Directorate of Sericulture, Khanapara, and Guwahati, Assam. The larvae were maintained on the fresh leaves of *Persea bombycina* (Family: Lauraceae Vernacular name: Som) serving as the exclusive food source throughout the experiment period. The rearing environment was maintained within optimal parameters for silkworm development, with temperature regulated at 20-30 and relative humidity at 70-90% through natural ventilation. Standard silkworm rearing protocols were followed, including daily provision of fresh host plant leaves and regular sanitation measures to ensure hygiene.

2. Selection of causative agents for silkworm infection:

The potential pathogenic agents were selected based on their ability to induce diseases in silkworm. Hence, two fungal and two bacterial species were chosen as representatives according to the previously available literature. The fungal species *Beauveria bassiana* (Saharia *et al.*, 2023) [36] and the bacterial species are *Bacillus thuringiensis* (Haloi *et al.*, 2016) [19] and *Serratia marcescens* (Zhang *et al.*, 2020) [42].

The bacterial strains *B. thuringiensis* (MTCC 1953) and *S. marcescens* (MTCC 86), along with the fungal strain *B. bassiana* (MTCC 2028), were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. All selected microbial cultures were maintained on both liquid (Nutrient broth & PDA) and solid (Nutrient agar & PDA) media. For long-term use and consistency, these cultures were preserved and sub cultured at regular intervals for 15-20 days.

3. Immunization of larvae

Fifth instar Muga silkworms (*Antheraea assamensis*) were divided into five groups with 20 larva each for immunization. The control group received 10 μ l of Phosphate-Buffered Saline (PBS), while the remaining four groups were immunized with 10 μ l suspensions. The immunization procedure was carried out using a sterile 1ml ultra-fine disposable insulin syringe (31GX5/16-inch needle, Becton, Dickinson and company) following the method described by Haloi *et al.*, (2016) [19]. *B. thuringiensis* and *S. marcescens* cultures were diluted in PBS to achieve the final concentration of 1×10^8 CFU/ml (Haloi *et al.*, 2016) [19]. *B. bassiana* suspension was prepared by harvesting conidia from a 7-day-old culture grown on potato dextrose agar (PDA). The conidial concentration was adjusted to 1×10^8 conidia/ml using sterile distilled water containing 0.05% Tween-80. All injections were administered sub-dorsally between the prolegs of larvae. Post-injection, the larvae were maintained in separate rearing containers under standard laboratory conditions ($25 \pm 2^\circ\text{C}$, 70–75% Relative Humidity) and were monitored regularly for signs of pathogenic symptoms.



Fig 1: Process of injection into silkworm for induction of pathogenicity

4. Collection of Haemolymph

Haemolymph samples were collected from the immunized and control larvae after 24 hours of treatment (Fig1). Before haemolymph collection, the silkworms were sterilized and the haemolymph was collected by making a small incision at the last abdominal leg of each larva. To preserve sample integrity, pre-chilled Eppendorf tubes containing a pinch of Phenyl Thio Urea (PTU) were used to prevent protein oxidation and degradation. The collected haemolymph was mixed thoroughly and centrifuged at 3000rpm for 15 minutes at 4 to separate the haemocytes. After centrifugation, the pellet was discarded, and the cell-free supernatant was stored at -20 until further analysis (Nayak *et al.*, 2018) [31].

5. Preparation of methanolic hemolymph extracts

Low molecular weight anti-microbial peptides were isolated from the hemocyte free hemolymph by the standard extraction method (Schoofs *et al.*, 1990). The hemolymph was diluted 10 times with the extraction solution with methanol: glacial acetic acid: water (90:1:9) and mixed thoroughly. The solution was centrifuged at 20,000 g for 20 minutes at 4 $^\circ\text{C}$. The obtained supernatant was collected,

vacuum dried and the pellet was stored at -20°C until needed. Before use, it was dissolved in an appropriate volume of sterile distilled water. The hemolymph extract obtained contained proteins and peptides of molecular weight below 30 kDa.

6. Antimicrobial assay of the hemolymph extracts

6.1 Media Preparation

The Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were prepared following the standard methods. The prepared media were autoclaved at 121°C for 15 minutes at 15 psi. Nutrient Agar (NA) was used as the culture medium for bacterial strains, and Potato Dextrose Agar (PDA) was used for fungal strains.

6.2 Well diffusion method

The antimicrobial activity of haemolymph extracted from silkworms infected with different microbial pathogens such as *Bacillus thuringiensis*, *Serratia marcescens* and *Beauveria bassiana* was tested using the well diffusion method (Eloff, 2004) [14]. Each culture plate was poured with the respective sterilized media and allowed to solidify. After solidification, five wells were aseptically made in each plate using a sterile cork borer. The central well was filled with phosphate-buffered saline (PBS) as a negative control. The remaining three wells were filled with haemolymph samples collected from silkworms infected with each of the pathogens. The plates were incubated at 37°C for 24 hours for bacterial strains and at 28°C for 48–72 hours for fungal strains. Post incubation, the plates were examined for clear zones of inhibition around the wells to assess the rate of effectiveness of the haemolymph against the test organisms.

7. Selection of plant species

To evaluate the antimicrobial efficacy of plant-derived essential oils against microbial pathogens affecting *A. assamensis*, three medicinally important plant species were selected, which are *Cinnamomum verum*, *Litsea salicifolia*, and *Andrographis paniculata*. The selection of these plant species was based on their well-documented antimicrobial properties, which have been reported in earlier studies (Sharma *et al.*, 2020; Govindarajan *et al.*, 2023) [16, 39].

7.1 Extraction of essential oil

The selected plant species *Litsea salicifolia*, *Cinnamomum verum*, and *Andrographis paniculata* fresh and healthy leaves were collected, shade-dried, and coarsely powdered prior to the extraction process. We have taken leaves for *L. salicifolia*, bark for *C. verum* and leaves and stem for *A. paniculata*. The essential oils were extracted using Soxhlet apparatus. 10 grams of dried and powdered sample of each plant material was weighed accurately and loaded into a thimble placed inside the main chamber of the Soxhlet extractor (Bontzolis *et al.*, 2024) [6]. Organic solvent hexane was used as the extraction solvent, due to its non-polar nature and high efficacy in dissolving essential oils and other lipophilic compounds. The extraction process was allowed to proceed for approximately 6–8 hours, ensuring extensive extraction of oil components. The ratio of the plant material to solvent ratio was 1:10 (w/w). Extractions were done varying from 360 to 720 min, in duplicates. The extraction temperature was set to 68°C for hexane. The solvent from the collected extract was then removed using a rotary evaporator under reduced pressure at low temperature (approximately $40\text{--}45^{\circ}\text{C}$) to avoid the degradation of

compounds. The extracted oils were collected in clean, amber-coloured glass vials, sealed, and stored at 4°C in a refrigerator to prevent oxidative degradation and maintain their bioactive properties until further use in antimicrobial bioassays. The yield of the essential oil from each plant was recorded, and the oils were visually inspected for physical characteristics such as colour, consistency, and aroma.

7.2 Essential oil analysis by GC-MS

To determine the bioactive constituents, present in the essential oils of selected plant species (*C. verum*, *L. salicifolia*, and *A. paniculata*), and Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted. The GC-MS analysis was performed using the standard protocol described by Adams *et al.*, (2007) [11] with slight modification. The analysis was carried out using a PerkinElmer Clarus680 GC/600C MS system. The carrier gas used was helium, with a constant flow rate of 1.0 ml/min. The temperature was programmed from 60°C (held for 3 minutes) to 280°C at a rate of $10^{\circ}\text{C}/\text{min}$. The identification of compounds was done by comparing the mass spectra with NIST Library.

7.3 Assessment of Antimicrobial Activity of Essential oils

7.3.1 In vitro assessment

The antimicrobial activity of selected plant essential oils was evaluated against potential pathogens of *A. assamensis* such as bacterial species *S. marcescens* and *B. thuringiensis* and the fungal species *B. bassiana*. The assessment was carried out using the well diffusion method mentioned earlier in the section 2.6.2. The essential oil concentration of 3% was selected for the assessment according to earlier literature (Saad *et al.*, 2019) [35]. Each essential oil was tested individually against each microorganism on separate Petri dishes. The microorganisms were spread evenly across the surface of the solidified media using a sterile cotton swab. The wells (approximately 6 mm in diameter) were bored into the agar using a sterile cork borer. Four wells were created on each plate in an anticlockwise arrangement taking Phosphate buffered saline (PBS) as control well. After incubation, the zones of inhibition were measured (in mm) using a transparent ruler. Each test was performed in triplicate to ensure reproducibility and accuracy of results.

7.3.2 In vivo assessment

For *in vivo* assessment of the essential oils, the silkworms were fed the som leaves by spraying the oils (3%) (Saad *et al.*, 2019) [35] On it. Live and dead silkworms were counted to know the effectiveness of the treatment.

8. Statistical analysis

All the experiments were done on triplicates. Statistical analysis was done on Microsoft Excel-2019 software.

Results and Discussions

1. Antimicrobial Assay of Haemolymph extract from Infected Silkworms

In the present study antimicrobial activity of antimicrobial peptides present in the haemolymph extracted from silkworms infected with different pathogens was tested against *B. thuringiensis*, *S. marcescens*, *B. bassiana* respectively using the well diffusion method (Figure 2).

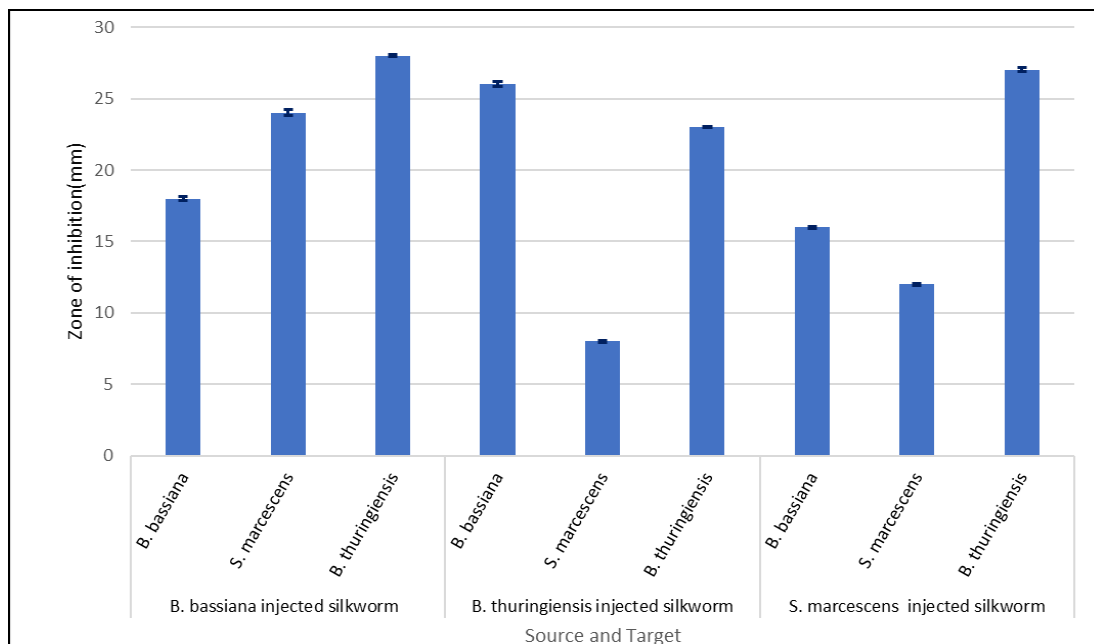


Fig 2: Zone of inhibition of each of the pathogen plates formed by *B. bassiana*, *B. thuringiensis* and *S. marcescens* injected silkworm haemolymph extract (Zone of inhibition \pm SD).

The haemolymph from the treated silkworms were collected and screened for their activity against the pathogens themselves (Figure 2). *B. bassiana* injected silkworm haemolymph showed highest activity against *B. thuringiensis*. Similarly, *B. thuringiensis* injected silkworm showed highest activity against *B. bassiana* and *S. marcescens* showed highest activity against *B. thuringiensis*. Earlier works showed significant effect of microbe induced antimicrobial peptides on inhibiting the bacterial and fungal infections in insects (Scieuzo *et al.*, 2023) [37].

2. GC-MS analysis of plant essential oils

Gas Chromatography–Mass Spectrometry (GC-MS) was employed to characterize the chemical constituents of essential oils extracted from *A. paniculata*, *L. salicifolia*, and *C. verum* (Figure 3).

The essential oil extracted from *A. paniculata* revealed the presence of two significant antimicrobial components such as Isopropyl phenyl ketone and Dimethylsulfoxonium Formylmethylide. Both chemical components have good antimicrobial activity against pathogens (Bashir and Kumar, 2021; Jha *et al.*, 2022) [5, 23].

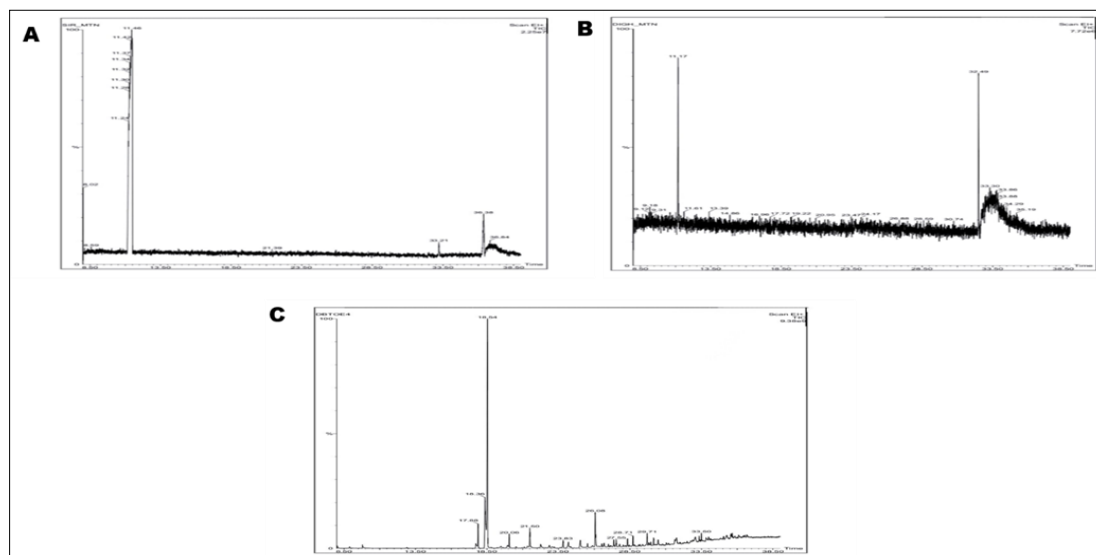


Fig 3: GC-MS spectra of A) *A. paniculata* B) *L. salicifolia* and C) *C. verum*

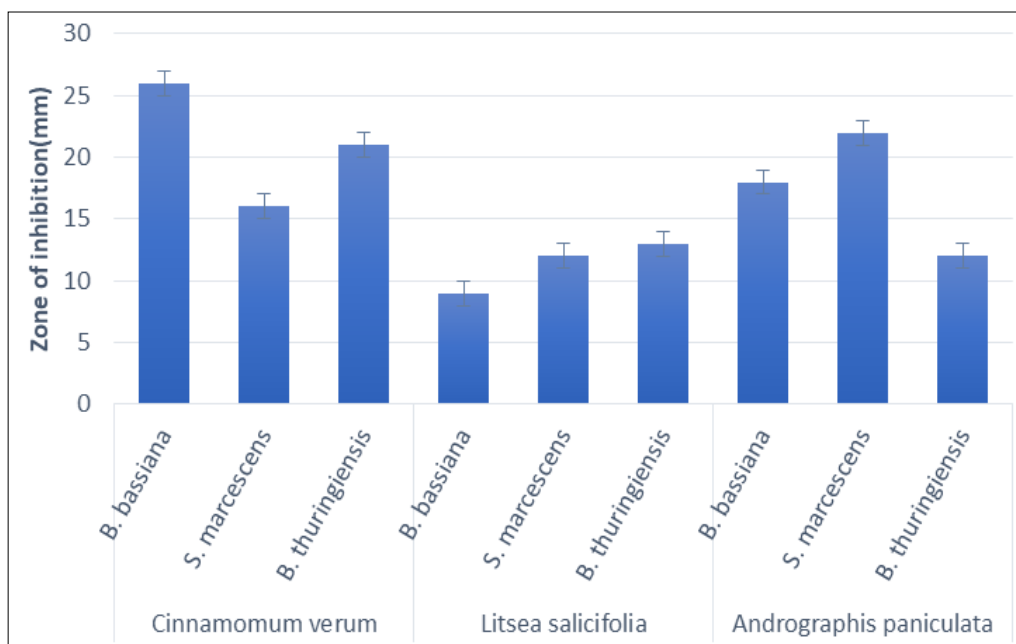
Another plant *L. salicifolia* which is a secondary host plant of Muga silkworm showed Benzyl benzoate and Cinnamic Acid as main peak are very significant compounds. Benzyl benzoate is a very good antimicrobial agent which was reported to be effective against *Escherichia coli*, *Enterococcus aerogenes*, *Bacillus cereus* and *Staphylococcus aureus* (Diastuti *et al.*, 2020) [11]. Cinnamic

acid is also a potent antimicrobial agent (Guzman, 2014) [18]. *C. verum* showed six significant compounds such as Hexanedioic acid, Cinnamaldehyde, Coumarin, Longifolene, Pentanedioic acid and Dodecane (Liao *et al.*, 2025; Ahmed *et al.*, 2024; Mukai *et al.*, 2018; El Hachlafi *et al.*, 2023; Doyle & Stephens, 2019; Chu *et al.*, 2014; Padma *et al.*, 2019) [2, 8, 12, 13, 27, 30, 32].

Table 1: Chemical compounds found in the GC-MS analysis of *A. paniculata*, *L. salicifolia*, and *C. verum*.

| Plant Name | Retention Time (RT) | Metabolites | M/W | Relative abundance |
|-----------------------|---------------------|-------------------------------------|-----|--------------------|
| <i>A. paniculata</i> | 33.208 | Isopropyl phenyl ketone | 148 | 1.50 |
| | 11.375 | Dimethylsulfoxonium Formylmethylide | 120 | 100 |
| <i>L. salicifolia</i> | 11.175 | Benzyl benzoate | 300 | 76.24 |
| | 32.498 | Cinnamic Acid | 299 | 76.24 |
| <i>C. verum</i> | 18.538 | Hexanedioic acid | 174 | 100.00 |
| | 21.504 | Cinnamaldehyde | 134 | 9.72 |
| | 26.076 | Coumarin | 146 | 16.35 |
| | 29.707 | Longifolene | 204 | 5.40 |
| | 18.358 | Pentanedioic acid | 174 | 39.71 |
| | 23.830 | Dodecane | 188 | 3.80 |

3. Antimicrobial activity of plant based essential oils against silkworm pathogens

**Fig 4:** Antimicrobial Activity of Plant-Based Essential Oils against Pathogens

Essential oils from the plant materials were extracted and screened for their activity against the pathogens (Figure 4). Oil extracted from *C. verum* showed highest antimicrobial activity compared to *L. salicifolia* and *A. paniculata*. *C. verum* oil exhibited highest activity against *B. bassiana* injected silkworms. While *L. salicifolia* oil showed the lowest activity against *B. bassiana* injected silkworms. *A. paniculata* oil displayed highest zone of inhibition against the *S. marcescens* injected silkworms. Earlier reports on the different compounds found in these plants showed very good activities against the bacterial and fungal pathogens (Liao *et al.*, 2025; Ahmed *et al.*, 2024; Mukai *et al.*, 2018; El Hachlafi *et al.*, 2023; Doyle & Stephens, 2019; Chu *et al.*, 2014; Padma *et al.*, 2019) [2, 8, 12, 13, 27, 30, 32]. These studies advocate the importance of these plant essential oils against microorganisms and as a potential pathogen killer of the Muga silkworms.

4. Essential oil treatment on pathogen-infected silkworms

The essential oils from all three selected plants were sprayed on the different groups of the silkworms to study their effect on the survival after 24 and 48 hours of treatment. A 3% essential oil was sprayed on them taking Phosphate buffered

saline (PBS) as a control. In every group, a greater number of silkworms survived than the control group, which suggested the effectiveness of the essential oil treatments.

Conclusions

The plant extracts containing bioactive metabolites of medicinal plants *A. paniculata*, *L. salicifolia* and *C. verum* exhibited high potential to inhibit such pathogens. A variety of metabolites such as cinnamic acid, cinnamaldehyde, Coumarine, Benzene benzoate, Hexanedioic acid, Dodecane, Pentadecanoic acid, Longifolin are some major compounds were extracted and identified from the plants were responsible for antimicrobial activity of the plant extracts against the pathogen of Muga silkworm diseases. However, the antimicrobial peptide extracted from the hemolymph of infected silkworm provides immunity against the pathogens tested in the present study. Thus, it can be concluded that the extract of medicinal plants used under this study could be used as biopesticide to control the disease of muga silkworms.

Acknowledgement

We thank Department of Biotechnology, India for providing the DBT-NER Biotech Hub grant for this research work.

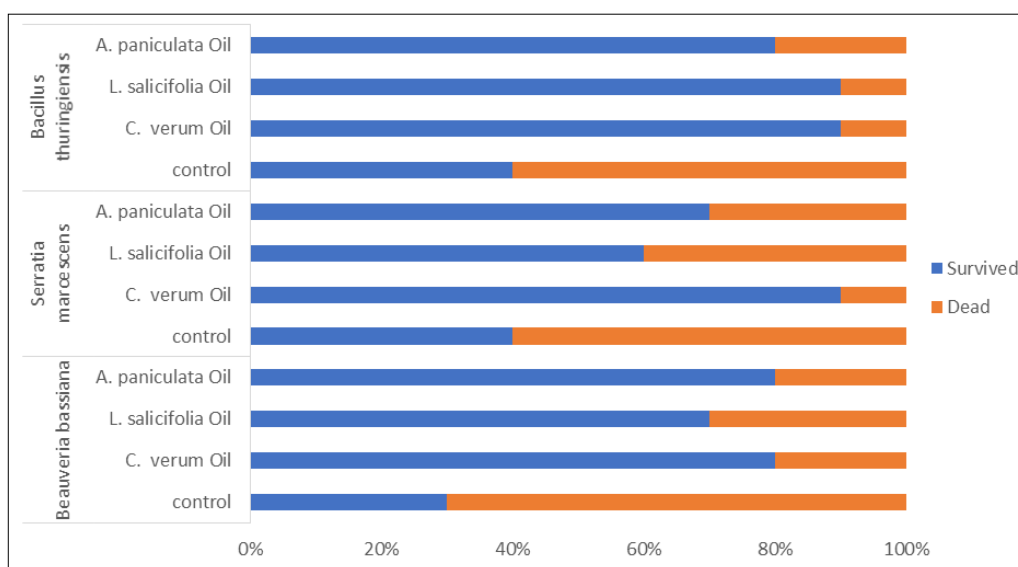


Fig 5: Percentage of survived and dead silkworms after 24 hours of essential oil treatment on pathogen-infected silkworms.

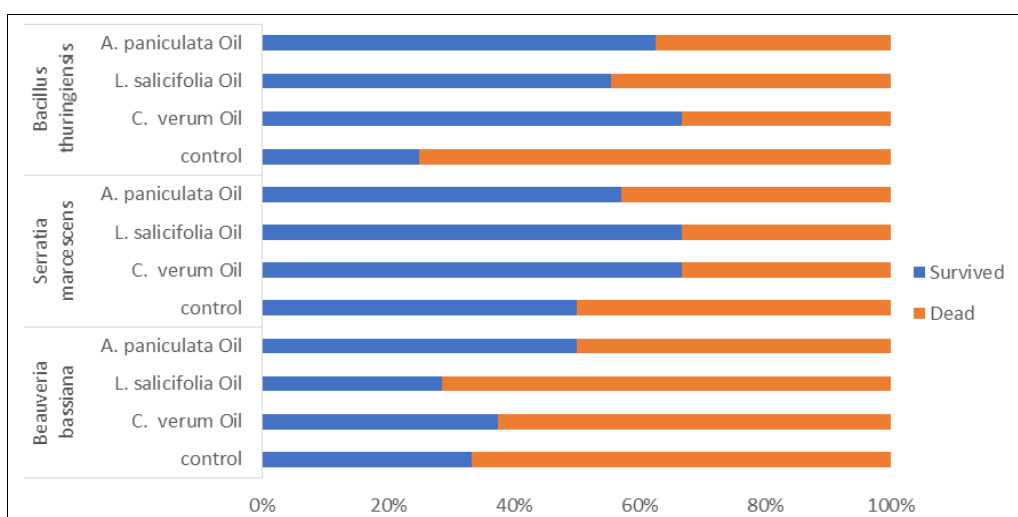


Fig 6: Percentage of survived and dead silkworms after 48 hours of essential oil treatment on pathogen-infected silkworms.

References

- Adams RP, Beauchamp PS, Dev V, Dutz SM. New natural products isolated from one-seeded *Juniperus* of the Southwestern United States: isolation and occurrence of 2-ethenyl-3-methyl phenol and its derivatives. *Journal of Essential Oil Research*,2007;19(2):146-152.
- Ahmed GE, Elshahid ZA, El-Sawy ER, Abdel-Aziz MS, Abdel-Aziem A. Synthesis, biofilm formation inhibitory, and inflammation inhibitory activities of new coumarin derivatives. *Scientific Reports*,2024;14(1):9106.
- Al-Garadi MA, Qaid MM, Alqhtani AH, Alhajj MS, Al-Abdullatif AA, Al-Mufarrej SI. *In vitro* antimicrobial efficacy assessment of ethanolic and aqueous extracts of cinnamon (*Cinnamomum verum*) bark against selected microbes. *Brazilian Journal of Poultry Science*,2023;25:2022.
- Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—a review. *Food and chemical toxicology*,2008;46(2):446-475.
- Bashir SF, Kumar G. Preliminary phytochemical screening and *in vitro* antibacterial activity of *Plumbago indica* (Laal chitrak) root extracts against drug-resistant *Escherichia coli* and *Klebsiella pneumoniae*. *Open Agriculture*,2021;6(1):435-444.
- Bontzolis CD, Dimitrellou D, Plioni I, Kandyli P, Soupioni M, Koutinas AA, *et al.* Effect of solvents on aniseed aerial plant extraction using soxhlet and ultrasound methods, regarding antimicrobial activity and total phenolic content. *Food Chemistry Advances*,2024;4:100609.
- Chao WW, Lin BF. Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). *Chinese medicine*,2010;5(1):17.
- Chu KB, Jeon GC, Quan FS. Hexanedioic acid from *H ermetia illucens* larvae (*D iptera: S tratiomyidae*) protects mice against *K lebsiella pneumoniae* infection. *Entomological Research*,2014;44(1):1-8.
- Dash S, Anas M, Gowrisankar R, Dasari S. *Bio-Nanotechnological Advances in Sericulture: Applicability of Micrococoon and Silk Proteins*, 2023.
- Devi B, Chutia M, Bhattacharyya N. Food plant diversity, distribution, and nutritional aspects of the endemic golden silk producing silkworm, *Antheraea*

- assamensis*—a review. *Entomologia Experimentalis et Applicata*,2021:169(3):237-248.
11. Diastuti H, Chasani M, Suwandri S. Antibacterial activity of benzyl benzoate and crotepoide from *Kaempferia rotunda* L. Rhizome. *Indonesian Journal of Chemistry*,2020:20(1):9-15.
 12. Doyle AA, Stephens JC. A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia*,2019:139:104405.
 13. El Hachlafi N, Mrabti HN, Al-Mijalli SH, Jeddi M, Abdallah EM, Benkhaira N, *et al.* Antioxidant, volatile compounds; antimicrobial, anti-inflammatory, and dermatoprotective properties of *cedrus atlantica* (endl.) manetti ex carriere essential oil: *in vitro* and *in silico* investigations. *Molecules*,2023:28(15):5913.
 14. Eloff JN. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation, 2004.
 15. Goswami D, Rabha B, Veer V. Muga Silk-The Golden Thread of Assam. In *Bioprospecting of Indigenous Bioresources of North-East India*. Singapore: Springer Singapore, 2016, 279-294.
 16. Govindarajan N, Somasundaram L, Duraipandian C, Punnose AM. Unveiling the Ethnopharmacological and Phytopharmacological Potential of *Litsea salicifolia* (*Lauraceae*). *Systematic Reviews in Pharmacy*, 2023, 14(11).
 17. Guo J, Yan S, Jiang X, Su Z, Zhang F, Xie J, *et al.* Advances in pharmacological effects and mechanism of action of cinnamaldehyde. *Frontiers in pharmacology*,2024:15:1365949.
 18. Guzman JD. Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity. *Molecules*,2014:19(12):19292-19349.
 19. Haloi K, Kalita MK, Nath R, Devi D. Characterization and pathogenicity assessment of gut-associated microbes of muga silkworm *Antheraea assamensis* Helfer (*Lepidoptera: Saturniidae*). *Journal of invertebrate pathology*,2016:138:73-85.
 20. Hou C, Qin G, Liu T, Geng T, Gao K, Pan Z, *et al.* Transcriptome analysis of silkworm, *Bombyx mori*, during early response to *Beauveria bassiana* challenges. *PLoS One*,2014:9(3):91189.
 21. Ishii K, Adachi T, Hara T, Hamamoto H, Sekimizu K. Identification of a *Serratia marcescens* virulence factor that promotes hemolymph bleeding in the silkworm, *Bombyx mori*. *Journal of invertebrate pathology*,2014:117:61-67.
 22. Isman MB. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochemistry reviews*,2020:19(2):235-241.
 23. Jha V, Risbud A, Matharoo DK, Preman G, Thube S, Bhosale A, *et al.* Chemical composition and antimicrobial activity of essential oil obtained from *Jasminum Sambac*. *J. Plant Biol. Crop. Res*,2022:5:1065.
 24. Jigyasu DK, Patidar OP, Kumar A, Shabnam AA. Muga silk insect (*Antheraea assamensis*). In *Commercial Insects*. CRC Press, 2023, 126-145.
 25. Kalita N, Kalita MC, Banerjee M. Reactive oxygen species generation in the antibacterial activity of *litsea salicifolia* leaf extract. *Int J Pharm Pharm Sci*,2016:8:189-93.
 26. Kumar V. The scanning electron microscopic study of the infection and conidial development of *Aspergillus tamarii* Kita on its host, the silkworm, *Bombyx mori* Linn. *Central European Journal of Biology*,2007:2(4):574-587.
 27. Liao Z, Zhu X, Parumasivam T, Xiao Z, Alrosan M, Alu'datt MH, *et al.* Synergistic Effects of Glutaric Acid With Selected Synthetic Preservatives on the Shelf Life of Refrigerated Beef. *Journal of Food Biochemistry*,2025:2025(1):5021230.
 28. Luo X, Ma Y, Hu Y, Ma H, Yu J, Liu L, *et al.* Preparation and characterization of silk fibroin-sericin composite material through a one-pot method. *Polymer*,2025:322:128149.
 29. Mishra SK, Sangwan NS, Sangwan RS. Phcog rev.: Plant review *Andrographis paniculata* (Kalmegh): A review. *Pharmacognosy Reviews*,2007:1(2):283-298.
 30. Mukai A, Takahashi K, Ashitani T. Antifungal activity of longifolene and its autoxidation products. *European Journal of Wood and Wood Products*,2018:76(3):1079-1082.
 31. Nayak T, Mandal SM, Neog K, Ghosh AK. Characterization of a gloverin-like antimicrobial peptide isolated from muga silkworm, *Antheraea assamensis*. *International Journal of Peptide Research and Therapeutics*,2018:24(2):337-346.
 32. Padma M, Ganesan S, Jayaseelan T, Azhagumadhavan S, Sasikala P, Senthilkumar S, *et al.* Phytochemical screening and GC-MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L), 2019.
 33. Pu S, Ma H, Deng D, Xue S, Zhu R, Zhou Y, *et al.* Isolation, identification, and characterization of an *Aspergillus niger* bioflocculant-producing strain using potato starch wastewater as nutilite and its application. *PLoS one*,2018:13(1):0190236.
 34. Puppala ER, Abubakar M, Swamy V, Yalamarthy SS, NP S, Prakash AN, *et al.* *Litsea salicifolia* Roxb. bark ethanolic extract attenuates rheumatoid arthritis by inhibiting TLR4/NF-κB and activating SIRT1-Nrf2/HO-1 signaling axis: In-vitro and in-vivo evaluations. *Phytomedicine Plus*,2023:3(1):100390.
 35. Saad MS, Elyamani EM, Helaly WM. Controlling of bacterial and fungal diseases that contaminating mulberry silkworm, *Bombyx mori* by using some plant extracts. *Bulletin of the National Research Centre*,2019:43(1):172.
 36. Saharia S, Kalita S, Kalita D, Ojah A, Bardoloi S. Assessment of the Effect of Methanolic Herbal Extract on Cocoon Parameters and Tensile Properties of Silk Fiber Spun by *Beauveria bassiana* Infected Muga Silkworm, *Antheraea assamensis* Helfer. *Asian Journal of Biological and Life Sciences*,2023:12(2):395.
 37. Scieuzo C, Giglio F, Rinaldi R, Lekka ME, Cozzolino F, Monaco V, *et al.* *In vitro* evaluation of the antibacterial activity of the peptide fractions extracted from the hemolymph of *Hermetia illucens* (*Diptera: Stratiomyidae*). *Insects*,2023:14(5):464.
 38. Seyed Ahmadi SG, Farahpour MR, Hamishehkar H. Topical application of *Cinnamon verum* essential oil accelerates infected wound healing process by increasing tissue antioxidant capacity and keratin

- biosynthesis. The Kaohsiung journal of medical sciences,2019:35(11):686-694.
39. Sharma A, Sharma P, Thakur J, Murali S, Bali K. Viral diseases of Mulberry Silkworm, *Bombyx mori* L. A review. Journal of Pharmacognosy and Phytochemistry,2020:9(2):415-423.
 40. Tayal MK, Chauhan TPS. Silkworm diseases and pests. In Industrial entomology. Singapore: Springer Singapore, 2017, 265-289.
 41. Tikader A, Vijayan K, Saratchandra B. Muga silkworm, *Antheraea assamensis* (*Lepidoptera: Saturniidae*)-an overview of distribution, biology and breeding. European Journal of Entomology, 2013, 110(2).
 42. Zhang Y, Shang R, Zhang J, Li J, Zhu G, Yao M, *et al.* Isolation and identification of two *Serratia marcescens* strains from silkworm, *Bombyx mori*. Antonie van Leeuwenhoek,2020:113(9):1313-1321.
 43. Zhang Y, Shang R, Zhang J, Li J, Zhu G, Yao M, *et al.* Isolation and identification of two *Serratia marcescens* strains from silkworm, *Bombyx mori*. Antonie van Leeuwenhoek,2020:113(9):1313-1321.