

Bio-Efficacy of *Solanum torvum* (Sw.) and Molecular Docking Insights for Sustainable Pest Control against *Spodoptera litura* (Fab.)

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Research Article

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Abstract

The antifeedant, larvicidal and histological effects of *Solanum torvum* leaf extracts were investigated against *Spodoptera litura*. The study found that the *S. torvum* ethyl acetate leaf extract showed a significant antifeedant effect against *S. litura* of (86.16%) at 5%. Ethyl acetate extract showed a larvicidal activity against *S. litura* of 88.21% and the LC50 value was 2.05%. Exposure of larvae to ethyl acetate leaf extract resulted in significant histological damage, particularly affecting epithelial, goblet and digestive cells. The molecular docking observation of insect chitin protein was noted in campesterin resulting in -7.8 kcal/mole energy. The results suggest that the inclusion of these plant extracts in integrated pest management approaches can promote sustainable and environmentally friendly pest control methods in agriculture.

1. Introduction

The devastating effects of *Spodoptera litura*, commonly known as tobacco worm, on agricultural and vegetable crops have been observed in numerous countries such as India, China and Japan. This voracious pest poses a significant threat to crop yields and food security and requires extensive research efforts to understand its biology, behaviour and management strategies. The researchers found that a total of 181 plant species from 39 different families were affected (Yooboon et al., 2019). The use of various categories of chemical insecticides has emerged as the predominant approach to pest management and control. The indiscriminate use of synthetic compounds can lead to a number of dangerous consequences, including environmental pollution, insect species toxicity and genetic resistance, among others.

Plant extracts and secondary metabolites have emerged as promising alternatives to synthetic pesticides, offering potential solutions for sustainable pest management strategies. In recent years, there has been growing interest in the potential of essential oils as a viable alternative to synthetic pesticides (Vendan et al., 2019). The utilization of plant pesticides offers several notable benefits, primarily attributed to their swift degradation and minimal persistence and bioaccumulation within the ecosystem (Senthil Nathan, 2013).

Solanum torvum from the solanaceae family was used for this research. They are widespread in India, Malaysia, Pakistan, tropical America (Zubaida Yousafa et al., 2013). Furthermore, an extensive literature search revealed no scientific publications on pest management or control, although locals have long used this plant as food and medicine to combat intestinal parasite larvae and dental problems (Murugesan et al., 2021). In addition, it is used to treat contact and repellent activity against *Callosobruchus maculatus* (Murugesan et al., 2022) and larvicidal and adulticidal activity against *Aedes aegypti* (Murugesan et al., 2023; Murugesan et al., 2023). The present study was to evaluate the antifeedant, larvicidal and histological effects followed by *in silico* studies. The main phytochemicals used in the docking study were revived from the previous GC-MS analysis report (Murugesan et al., 2022). The insect chitin protein was used for the molecular docking studies.

2. Materials and Methods

2.1. Plant material

Solanum torvum leaves were collected from Kovilvenni, Thiruvavur, Tamil Nadu in June 2022. It was authenticated by a plant taxonomist from the Rev Dr. S. John Britto, Rapinat Herbarium, St. Joseph College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

2.2. Preparation of plant extract

The *S. torvum* leaves were collected, allowed to dry in the shade at room temperature, and then ground with a mixer grinder. For 48 h, 3 L (1:3 w/v) of hexane, ethyl acetate, and methanol were used to extract the powder (1 kilogram). Using Whatman No. 1 filter paper and a Buchner funnel, the extract was filtered. Using a rotary evaporator (Model RE 801, Yamato, Japan) at 40° C and reduced pressure, the filtrate was dried. According to Murugesan et al. (2023), the crude extracts were kept cold until needed.

2.3. Mass culture of *Spodoptera litura*

The *S. litura* culture was maintained in the research department of zoology and biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur. A sterile plastic container containing castor leaves and a 10% honey solution was used to maintain the emerging adults on cotton wool (Liu et al., 2015), which was inserted into a tiny glass cap for egg laying. For insects, culture was carried out at 25–27°C and a relative humidity of 60–70%.

2.4. Antifeedant activity

The antifeedant effect of the *S. torvum* leaves extracts was examined using the leaf disc no-choice method (Isman et al., 1997). Different concentrations of crude extracts were prepared by dissolving in acetone and tested against *S. litura*. Fresh castor leaf discs with a diameter of 4 cm were punched out using a cork drill. They were then individually immersed in crude extract concentrations of 0.625, 1.25, 2.5, 5%. Since acetone was used to dissolve the crude extracts, the leaf discs dipped in the substance served as a negative control. To prevent the leaf discs from drying out too early, wet filter paper was placed in each 1.5 cm × 9 cm plastic petri dish and a single third instar larva was placed in each petri dish. The progressive leaf consumption of the treated and control larvae was recorded over 24 h using a leaf area meter (Delta-T Devices, S. No: 15736 F 96, UK). The number of leaves eaten by the treatment larvae was adjusted based on the negative control. For each treatment, five replicates each containing 10 larvae were kept.

$$\text{Antifeedant Index} = \frac{\text{Control} - \text{Treated}}{\text{Control} + \text{Treated}} \times 100$$

In the case of the control and treated discs, C and T denote the relative amounts of leaf consumed by the larvae.

2.5. Larvicidal Activity

Different concentrations of crude extracts were applied using the leaf dipping method. The larvae were exposed to the treated leaves. The larvae were kept alive on fresh castor bean leaves that had not been treated for a whole day. Fresh castor leaves were delivered every 24 h. Death of the larvae was noted 96 h after treatment. For each treatment, five replicates containing ten larvae each were maintained. The mortality percentage was calculated using Abbott (1925). The experiment was carried out with a light photoperiod of 14:10, a laboratory temperature of $27 \pm 2^\circ \text{C}$ and a relative humidity of $75 \pm 5\%$.

$$\text{Abbot corrected mortality} = \frac{\% \text{mortality in treatment} - \% \text{mortality in control}}{100 - \% \text{mortality in control}} \times 100$$

2.6. Histological analysis in midgut of *Spodoptera litura*

The digestive system of *S. litura* was used for the histological examination. The abdominal part of the larval parts was fixed in 10% neutral buffered formalin. The tissue samples were dehydrated and cleaned using a tissue processor. The samples were then fixed in paraffin blocks using a rotary microtome and an embedding station, 4 μm thick sections were cut (Leica RM2255 microtome, Buffalo Grove, IL, USA), and stained with hematoxylin and eosin stains. Finally, the stained sections of the tissue sample of *S. litura* larvae were examined using light microscopy (Suryani et al., 2020).

2.7. ADMET properties and Lipinski rule

A future molecular ligand molecule must reach its target at the correct concentration and remain stable in a bioactive state for a long period of time to be useful as a therapeutic component. This allows biological processes in the host body to occur to a predictable extent. The Swiss ADME online tool is considered a rapid and accurate model to explore the physicochemical properties, pharmacokinetics and drug-likeness of a target substance due to its application in ADME prediction research using the *in silico* approach.

2.8. Ligand preparation

The four phytochemicals were downloaded using PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). The Discovery Studio Visualizer was used to convert the 'sdf' files to 'pdb' format. The ligands were subjected to torsion adjustment. The 'pdbqt' file was created using the PyRx (Autodock Vina) utilities. Chemicals tested against insect chitin protein included myristic acid, geranylacetone, campesterol and linolenic acid. To simulate docking, the lowest energy ligands were loaded into AutoDock Vina.

2.9. Receptor preparation

The insect chitin protein is the subject of the present study. Downloads of the crystal structure of the insect chitin protein are available through the RCSB database at <https://www.rcsb.org/>. Changes to protein crystal structures include addition of charge, removal of water, and atomic substitution. Autodock 4.2 was used to create the 'pdbqt' file.

3. Molecular docking and determination of binding energy

Both the ligand and protein were prepared using AutoDock tool. The AutoDock Vina 4.2 assessment tool was used to determine the binding energies during docking. Among the several docking sites, the complex with the lowest energy consumption was selected and its analysis was carried out using the Discovery Studio Visualizer program.

3.1. Statistical analysis

The mortality data were subjected to one-way analysis of variance (ANOVA) with Tukey's multiple range tests to find the effective treatment at $P < 0.05$ and to determine the significant difference between treatments. Probit analysis was used to calculate the lethal concentration for 50% mortality (LC50) for a 96-h exposure period. Probit analysis was performed using SPSS statistical software (version 16.0).

4. Results

4.1. Antifeedant and larvicidal activity

The present study deals with the antifeedant and larvicidal activities of hexane, ethyl acetate, and methanol extracts of *S. torvum* against *S. litura*. Antifeedant activity of *S. torvum* crude extract was tested against *S. litura* results are presented in **Table 1**. The extract hexane and methanol were showed moderate antifeedant activity (51.33%), (77.16%) and ethyl acetate extract showed significant activity (86.16%) at 5% concentration. Ethyl acetate exhibited larvicidal activity of 88.21% with LC50 value of 2.05%. The larvae after treatment with ethyl acetate extract showed mortality in larval, and adult stages (**Table 2 and 3**)

4.2. Histological analysis in the midgut of *S. litura*

Histological analysis was observed 72 h after exposure in the midgut of 3rd instar *S. litura* larvae treated with a 5% concentration of *S. torvum* leaf extract. A significant effect was observed when the larvae were treated with a concentration of 5% of ethyl acetate extract. There was disruption of the striated border and peritrophic membrane of the epithelial cells. The maximum damage was observed in larvae exposed to ethyl acetate leaf extract. Epithelium and significant vacuolization in the goblet and digestive cells of the midgut (**Fig 1**).

4.3. ADME properties and Molecular docking analysis

The best docked ligand molecules were analysed based on their binding energy, type of hydrogen bond, and matching amino acid residues. Lipinski's rule has been used to evaluate phytochemical substances or ligands for their potential as drugs. Molecular properties observed included compound molecular weight, number of rotatable bonds, number of H-bond donors and acceptors, octanol-water partition coefficient (Log P), topological polar surface area (TPSA), and bioavailability for certain ligands. **Table 4** provides a clear representation of the physicochemical parameters used to assess the drug likeness of docked compounds.

Myristic acid was found to have a docking rank with scores of -5.1 (**Table 5**). Amino acids residues, such as HIS 74, GLN 88, LYS 80 and PHE 84 were found to be important in finding affinity for these molecules. The distances between these bonding contacts were measured to be HIS 74 (2.12 Å), GLN 88 (2.36 Å), and LYS 80 (2.74 Å) (**Fig 2**). Geranylacetone was found to have a docking score of -4.5 (**Table 5**). The amino acids residues, such as ASP 129, SER 138, and PRO 133 were found important for finding of affinities for these molecules. The distance of these covalent H-bond contacts ASP 129 (2.46 Å), SER 138 (1.87 Å), and PRO 133 (1.57 Å) as observed in **Fig 3**. Campesterin was found to have a best docking score of -7.8 (**Table 5**). The distances between these bonding contacts were measured to be 1.82 Å. The amino acids residues, such as ASN 95, LYS 96, and PRO 91 (**Fig 4**). A docking score of -4.0 was found for linolenic acid (**Table 4**). The distances between these bonding contacts were measured to be 1.16 Å to 2.34 Å. The amino acids residues, such as HIS 74, ASP 79, and PHE 84 (**Fig 5**).

5. Discussion and Conclusion

Many plants are reported to have antifeedant, growth inhibition, and larvicidal effects against *S. litura* such as, Eg., *Zingiber officinale*, *Pedaliium murex*, *Vitex negundo*, *Citrus sinensis* (Sahayaraj, 1998), *Melia dubia* (Koul et al., 2000), *Ocimum basilicum* (Pavela, 2004). Koul et al. (2004) found that the extract of *Aglaia elaeagnoidea* had maximum antifeedant and larvicidal activity against *Helicoverpa armigera* and *Pieris rapae*. *S. torvum* plant was studied with *C. quinquefasciatus* larvae achieved maximum larvicidal activity (Rahuman et al., 2008B). Pavunraj et al. (2011) stated that ethyl acetate leaf extract of *Pergularia daemia* has good antifeedant activity against *S. litura*. Salinas-Sanchez et al. (2012) reported that *T. erecta* hexane extract at 48%, acetone extract at 60% and ethanol extract at 500 ppm had a concentration of 72% compared to *S. litura* larvae. The results of Bhatt et al. (2014) were closely related to our result. It was found that *D. falcata* leaves had activity of 98.58%, *A. indica* fruit extract of 85.72% and *C. reflexa* extract of 98.58%.

The study's most important discovery was that the antifeedant treatment with the highest effectiveness also had the highest larvicidal activity. Many plants have been used for their larvicidal activity against *S. litura*, including *C. calamitosum*, *C. viscosum*, *C. multiflorum*, *C. philippinum*, *C. serratum*, *C. paniculatum* and *C. splendens* (Jadhav et al., 2016). These results support the validation of Jbilou et al. (2006) found that acetone and ethanol extracts of *Anticarsia gemmatalis* contained potential insecticidal agents to control *A. pubescens*. *Acorus calamus* ethyl acetate leaf extract at 5% concentration 40.24% and *Annona squamosa* at 40% larval mortality was achieved against *S. litura* (Raja et al., 2003). The leaf extract of *Marrubium vulgare* showed 42.2% larval mortality at a concentration of 5.0%. The larvae eventually stopped feeding, resulting in a developmental arrest at various stages of the larval instar (Pavela, 2004). The herbal insecticides and antifeedants can be important components of an integrated pest management program.

The peritrophic membrane of *S. litura* larvae remained intact in the midgut region and the untreated larvae showed signs of midgut architecture. Regenerative cells adhere to the surface of the basement membrane. Well-developed goblet cells and longitudinal muscle layers follow the epithelial cells, which

have an elongated nucleus and decondensed chromatin. The epithelial cells have a densely packed, striated brush border and form columnar epithelial cells (**Fig 1**). This condition shows that the proper functioning of the midgut cells enables the metabolic activity of the insect larvae (Suryani et al., 2020). The effect was found to be dose dependent. The current results were consistent with those reported by Fiaz et al. (2018) were found in the midgut digestive cells of *Anticarsia gemmatalis* and *S. litura* larvae.

In insects, digestion and food absorption take place in the midgut area, which is of endodermal origin (Caccia et al., 2019). The midgut is a crucial part of the alimentary canal and takes up a lot of space in the hemocoel. In addition, it plays a crucial role in many physiological controls such as blood flow, immunological response, and metabolism (Takeda, 2012). In addition, azadirachtin has been found to cause certain histological changes in the body tissues of insects (Rembold, 1991). When plant pesticides were applied to *Schistocerca gregaria* and *Locusta migratoria*, Cottee (1984) noted changes such as cell necrosis, vacuolization of the cytoplasm, reduction in nuclear size, and cell regeneration. Therefore, interfering with any of these processes can serve as a target or strategy for subsequent pest control measures.

Since chitin is a crucial component of the insect exoskeleton, chitin synthase has been discovered as a target protein for the production of insecticides control agents of insect pest, which are compounds intended to reduce insect pest populations (Rasoolizadeh A et al., 2016). Chitin synthase activity can be inhibited, leading to disruptions in chitin synthesis and exoskeleton assembly, potentially hindering insect pest growth and development. Chitin synthase is the target of many types of insecticides, such as ecdysone agonists and chitin synthesis inhibitors (Wang Y et al., 2020). Chitin synthesis inhibitors directly block the action of chitin synthase, while ecdysone agonists mimic the hormone ecdysone, which controls the exoskeleton rejection process in insects. Disruption to the regular molting process can hinder the growth and development of mosquitoes (do Nascimento ARB et al., 2022).

In conclusion, our research highlights the diverse potential of *S. torvum* leaf extracts and their transformative effect on the management of *S. litura* larvae. These findings not only expand our understanding of natural pest control, but also provide opportunities for the development of innovative, environmentally friendly solutions in the agricultural sector. We look forward to further research and application of these findings in the continued pursuit of sustainable pest control.

Declarations

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Authors Contribution

R. Murugesan: Writing – review & editing, Writing – original draft, Software, Methodology, Funding acquisition, Formal analysis, Conceptualization, Data curation, Investigation. **K. Vasuki:** Conceptualization, Data curation. **B. Kaleeswaran:** Investigation, Formal analysis, Methodology, Supervision, Validation.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Data availability

Open for all

Availability of data and material

All the collected articles and their data are enclosed in this article in the reference section.

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Tables

Table 1. Percent antifeedant activity of *S. torvum* leaf extracts against 3rd instar larvae of *S. litura* at different concentrations.

<i>Solanum torvum</i>		Dosage	Antifeedant
Leaf Extracts (%)	Hexane	0.625	04.52 ± 0.059 ^a
		1.250	10.70 ± 0.152 ^b
		2.500	20.83 ± 0.170 ^c
		5.000	51.33 ± 0.198 ^e
	Ethyl acetate	0.625	05.35 ± 0.093 ^a
		1.250	14.19 ± 0.654 ^b
		2.500	35.57 ± 0.400 ^c
		5.000	86.16 ± 0.133 ^e
	Methanol	0.625	05.22 ± 0.144 ^a
		1.250	13.87 ± 0.306 ^b
		2.500	31.15 ± 0.094 ^c
		5.000	77.16 ± 0.126 ^e

Each value is an mean of five replicates with standard error (Mean ± SE). Means within a column followed by the different letters are significantly different (P<0.05) as determined by Tukey's test.

Table 2. Larvicidal activity of 3rd instar larvae of *S. litura* exposed to *S. torvum* leaf extracts at different concentrations.

<i>Solanum torvum</i>		Dosage	Mortality
Leaf Extracts (%)	Hexane	0.625	04.18 ± 0.244 ^a
		1.250	12.87 ± 0.200 ^a
		2.500	24.86 ± 0.244 ^b
		5.000	52.21 ± 0.374 ^c
	Ethyl acetate	0.625	14.45 ± 0.244 ^a
		1.250	28.07 ± 0.374 ^b
		2.500	52.29 ± 0.200 ^c
		5.000	88.21 ± 0.200 ^e
	Methanol	0.625	06.28 ± 0.244 ^a
		1.250	18.11 ± 0.200 ^b
		2.500	34.38 ± 0.244 ^c
		5.000	68.11 ± 0.374 ^d

Each value is an mean of five replicates with standard error (Mean ± SE). Means within a column followed by the different letters are significantly different (P<0.05) as determined by Tukey's test.

Table 3. Probit analysis of larvicidal mortality for *S. litura* exposed to *S. torvum* leaf extracts at different concentrations.

<i>S. torvum</i> extracts	LC ₅₀ (%)	95% confidence limit (LL-UL)	LC ₈₀ (%)	95% confidence limit (LL-UL)	Slope ± SE	χ ² (df)
Hexane	5.025	4.306 - 6.162	-	-	1.99 ± 0.10	75.95 (23)
Ethyl acetate	2.05	1.83 - 2.29	4.61	3.99 - 5.53	2.39 ± 0.09	88.78 (23)
Methanol	3.31	2.98 - 3.72	-	-	2.33 ± 0.09	66.65 (23)

LC = lethal concentration; LL = lower limit; UL = upper limit; SE = standard error; χ² = chi-square; df = degree freedom.

Table 4. Drug-like parameters of the phytochemicals examined in this study

Ligands	MF	MW	Log P	NHD	NHA	Log	LV	RB
Myristic acid	C14H28O2	228.37	4.45	1	2	-3.35	0	12
Geranyl acetone	C13H22O	194.31	3.60	0	1	-4.8	0	6
Campesterin	C28H48O	400.68	6.29	1	2	-2.5	1	5
Linolenic acid	C18H30O2	278.43	0	1	2	0	0	13

MF- Molecular formula; MW- Molecular weight; NHD- Number of hydrogen bond donor; NHA- Number of hydrogen bond acceptor; Log P- Lipophilicity (Po/w); Log – Logarithmic skin permeation (Kp (cm/s); LV- Lipinski violation count; RB- Number of rotatable bonds.

Table 5. Molecular docking interacting result of bioactive compounds with insect chitin Protein

S. No	Compound Name	Docking score (kcal/mole)	RMSD (Å)	Bond length (Å)
1.	Myristic acid	-5.1	2.804	2.12, 2.36, 2.74
2.	Geranylacetone	-4.5	10.106	2.46, 1.87, 1.57
3.	Campesterin	-7.8	23.228	1.82
4.	Linolenic acid	-4.0	3.2	1.16 - 2.34

Figures

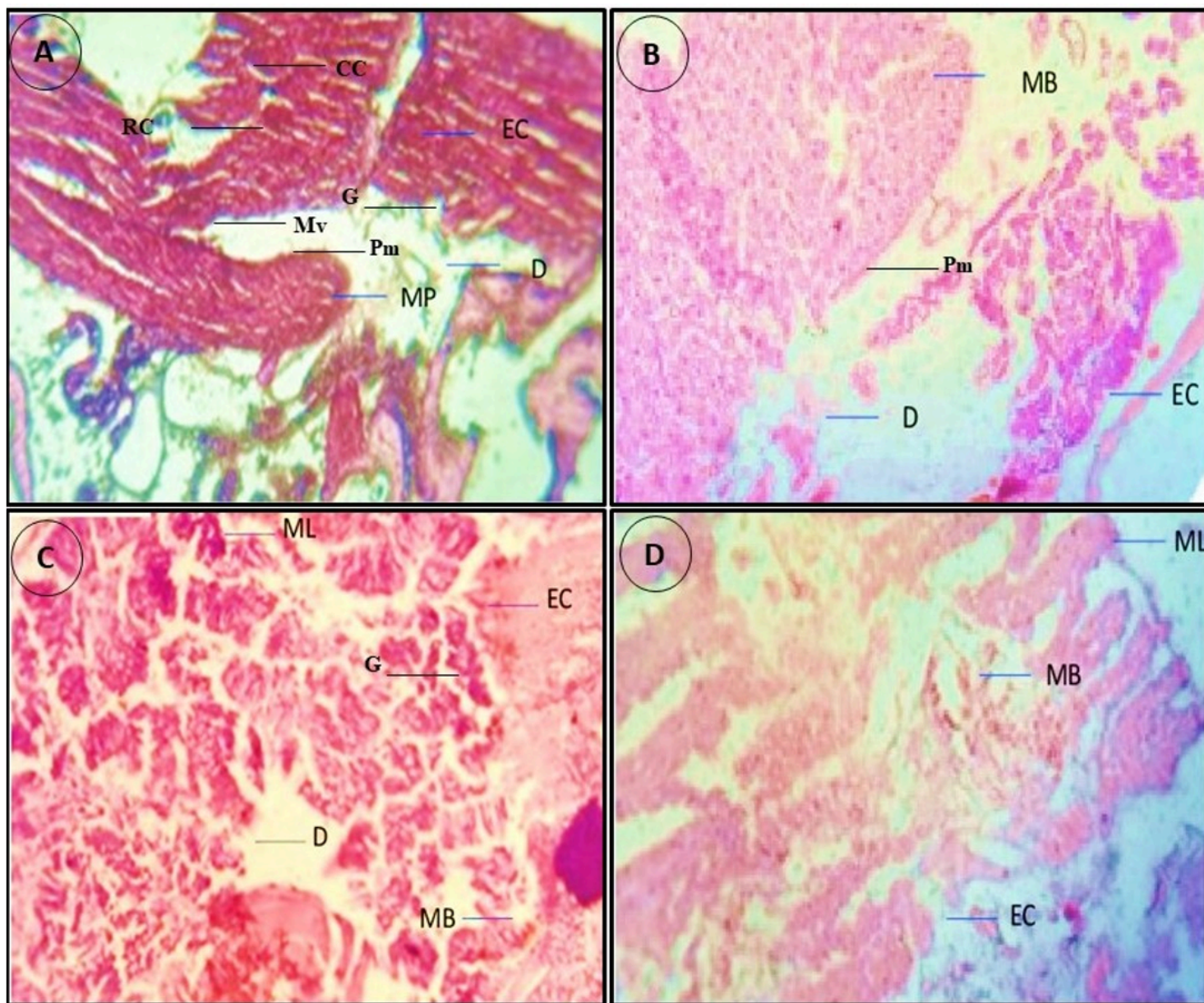


Figure 1

Histological examination of 3rd instar larvae of *S. litura* (A- control; B- hexane extract; C- ethyl acetate extract; D- methanol extract treatments). Muscular Layer (ML), Membrane Peritrophic (MP), Epithelial Cell (EC), Basal Membrane (MB) and disintegration (D), CC- Columnar cells, RC- Regenerative cells, G- Goblet cell, Mv- Microvilli, and Pm-Peritropic membrane.

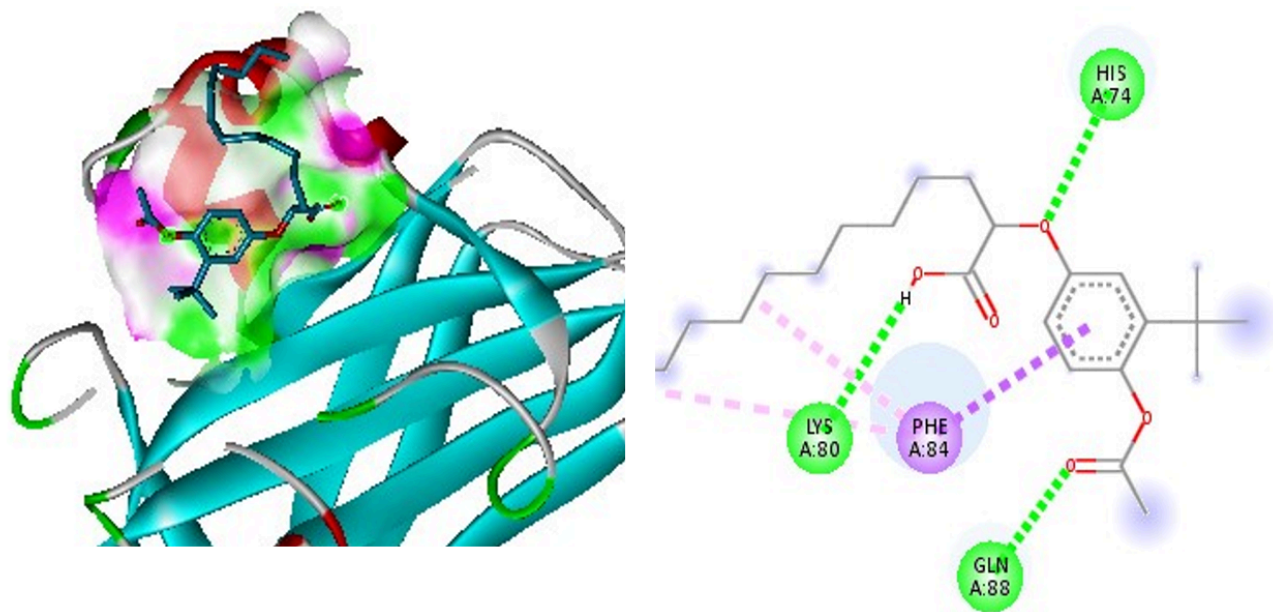


Fig. 2: a) Insect chitin protein 3D template reveals the interactions and binding interaction of protein and ligands; b) 2D template plots showing contacts between the functional groups of Insect chitin protein and the amino acids residues of Myristic acid.

Figure 2

See image above for figure legend.

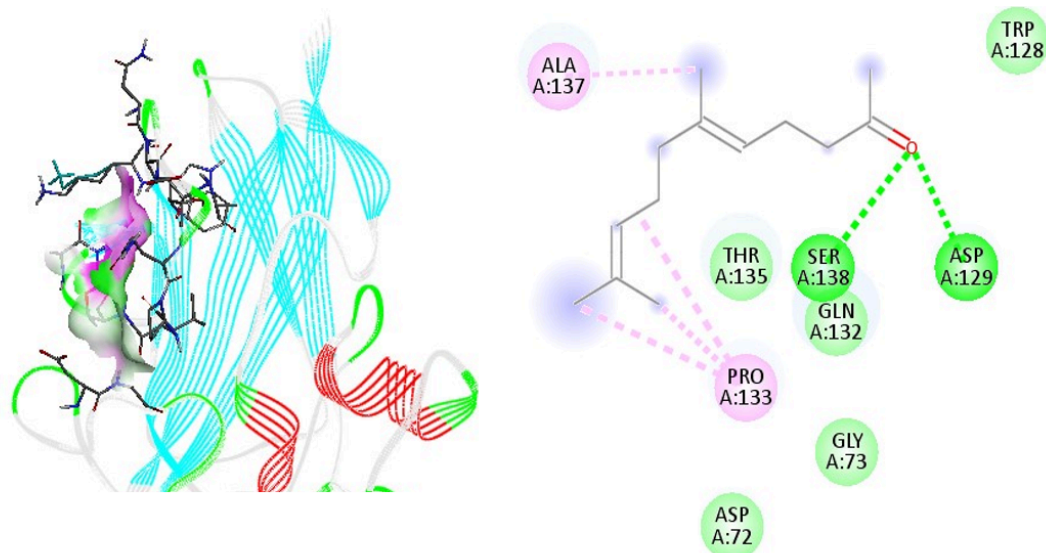


Fig. 3: a) Insect chitin protein 3D template reveals the interactions and binding interaction of protein and ligands; b) 2D template plots showing contacts between the functional groups of Insect chitin protein and the amino acids residues of Geranylacetone.

Figure 3

See image above for figure legend.

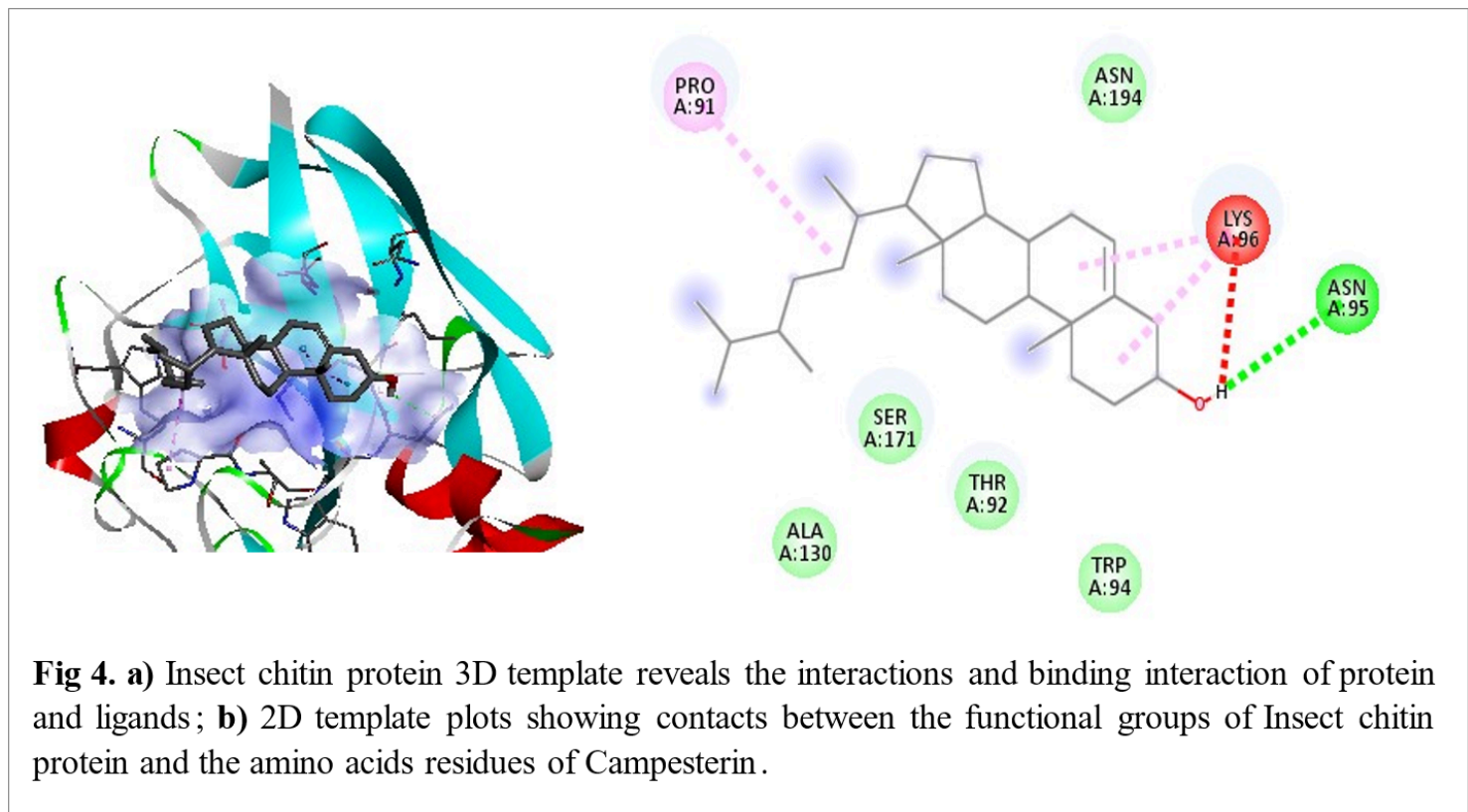


Figure 4

See image above for figure legend.

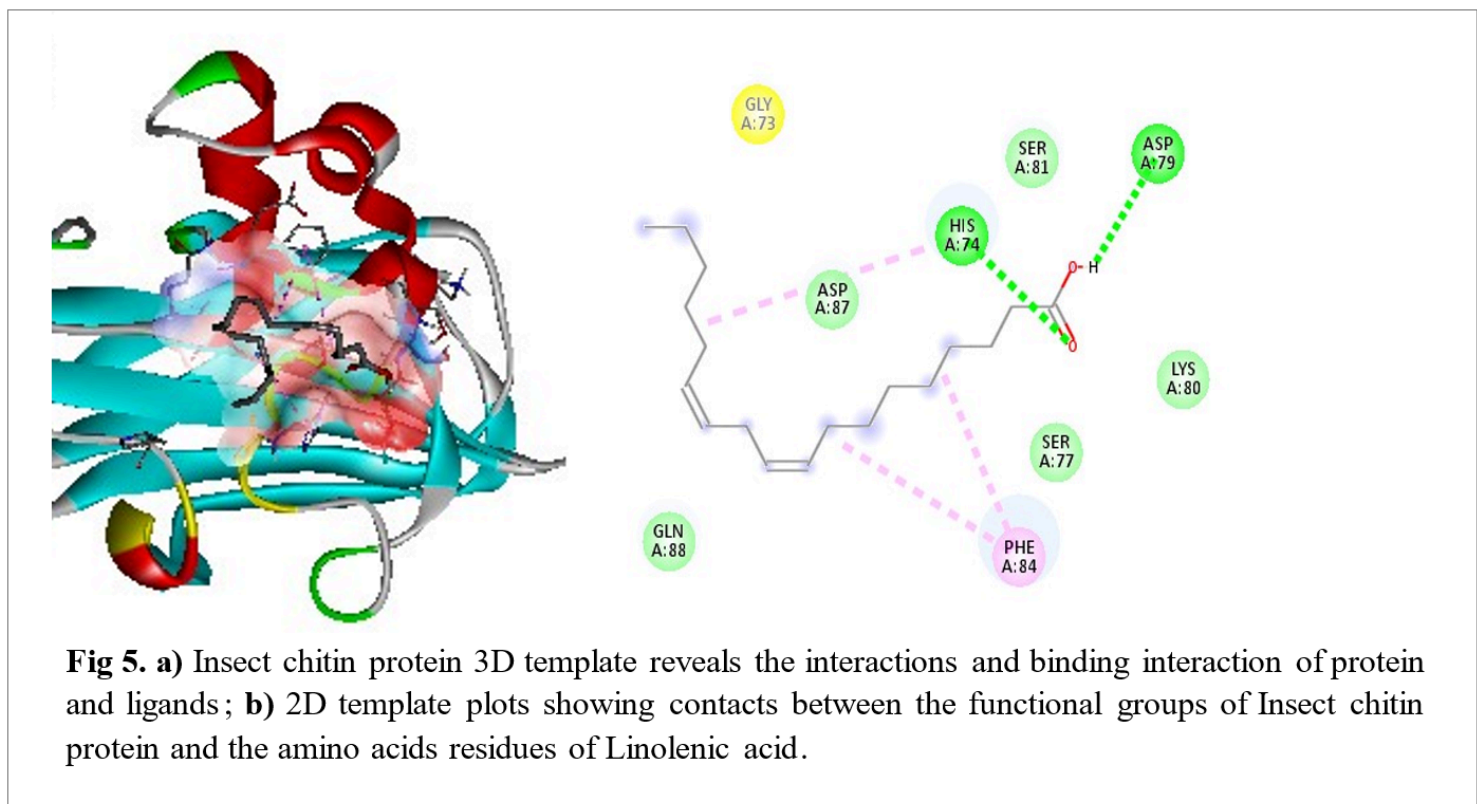


Figure 5

See image above for figure legend.

Supplementary Files

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- [GraphicalAbstarct.png](#)