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Insilico assessment of antiviral and antibacterial activity of some selected flavonoids against *Bombyx mori*

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Abstract
Flavonoids are a group of polyphenolic compounds distributed ubiquitously among many plants and have been reported to possess antimicrobial activity against a wide range of pathogens. Silkworm *Bombyx mori* is an important economic insect as it is used to convert leaf protein into silk whose production has an economic importance all over the world. However silkworm mortality is mainly caused by pathogenic microorganisms virus *Bombyx mori nuclear polyhedrosis virus* and bacteria *Staphylococcus aureus* thereby reducing the production of silk. In the current study, antibacterial and antiviral activity of the four flavonoids scandenone, tiliroside, quercetin-3,7-O-α-l-dirhamnoside, and kaempferol-3,7-O-α-l-dirhamnoside were studied using insilico techniques. Docking procedures were carried out using GOLD software and the results indicated that the flavonoids scandenone and quercetin-3,7-O-α-l-dirhamnoside possess good antimicrobial activity and could be promising leads for further drug development.

Keywords: Flavonoids, *Bombyx mori*, *Bombyx mori nuclear polyhedrosis virus*, Docking, GOLD.

1. Introduction
Flavonoids constitute a group of poly phenolic compounds, distributed universally among green plant kingdom occuring in free form, as glycosides, as well as methylated derivatives. They are responsible for colour and aroma of flowers, spore germination, growth and development of seedlings. They are obtained from the parent substances flavones found commonly in cell sap of young tissues of higher plants [1]. Over 5000 flavonoids have been isolated so far from fruits, vegetables, and beverages (e.g. wine and tea) derived from plants where they mostly found to occur as water-soluble glycosides [2]. (Kuhnau, 1976). There is increasing evidence that stress occuring due to biotic and abiotic factors could be protected by flavonoids [3]. Due to divergent biological activities which include antioxidant, anti-inflammatory, cardioprotective, antibacterial, antitumor, hepatoprotective, antiviral activities [4-12] exhibited by flavonoids, they have gained significant therapeutic importance.
The silkworm *Bombyx Mori* rearing is a traditional industry in Asia. Increase of larval growth and cocoon quality and quantity would result better economics for this industry, there by meeting the production needs. Silkworm mortality is mainly caused by pathogenic microorganisms, among which virus *Bombyx mori nuclear polyhedrosis virus (BmNPV)* and bacteria *Staphylococcus aureus* are affecting more. *Staphylococcus aureus* is a Gram-positive coccal bacterium that plays a major role in reducing the cocoon productivity both quantitatively and qualitatively. The virulence of *Staphylococcus aureus* was due to production of alpha haemolysin which is a major cytotoxic, small β-barrel pore-forming toxin that causes pore formation and cellular lysis in the susceptible organism. *Bombyx mori nuclear polyhedrosis virus (BmNPV)* is a circular double stranded DNA virus that causes deadly grasserie disease in silkworm there by effecting silkworm industry. In view of anti-infective properties [13] including antibacterial and antifungal activities [14] exhibited by flavonoids, an attempt has been made to study antiviral and antibacterial properties of flavonoids targeting the virus BmNPV as well as the bacteria *staphylococcus aureus*.
The aim of the present study was to elucidate the antiviral as well as antibacterial activities of the four flavonoids scandenone, tiliroside, quercetin-3,7-O-α-l-dirhamnoside, and kaempferol. Homology modelling of the Cysteine protease protein of the virus *Bombyx mori nuclear polyhedrosis virus (BmNPV)* was done using DS. After enery minimization, docking studies were performed with the selected flavonoids using GOLD software. As the x-ray crystal structure of the protein Alpha hemolysin of the bacteria *Staphylococcus aureus* was readily available (PDB ID: 3M2L), it was energy minimized and docking was performed with the four selected flavonoids.

2. Materials and Methods

Homology modeling of Cysteine protease protein structure of BmNPV.

Homology modeling which plays a crucial role in determining the structure of the proteins involves sequence based similarity search with known structures from PDB, structural alignment, three dimensional homology model building. All the above procedure was performed using Discovery studio.

Energy Minimization.

The modelled structure of Cysteine protease protein of BmNPV as well as Alpha hemolysin protein (PDB ID: 3M2L) of *Staphylococcus aureus* were energy minimized by employing CHARMm force fields and steepest descent algorithm followed by conjugant gradient algorithm in DS until the convergence gradient is satisfied.

Model Validation

RAMPAGE Server - Ramachandran Plot Analysis

Validation of protein structure was carried out using RAMPAGE Server [15]. It verifies parameters like Ramachandran plot quality, peptide bond planarity, bad nonbonded interactions, main chain hydrogen bond energy, C-alpha chirality and overall G factor, and the side chain parameters like standard deviations of chi1 gauche minus, trans and plus, and pooled standard deviations of chi1 with respect to refined structures.

Prosa.

This program [16, 17] compares Z scores between target and template structure and should be comparable.

RMSD.

Root Mean Squared Deviation (RMSD) which is used to represent the distance between two objects indicates that lower the RMSD value, more is the similarity between the structures. SPDBV program was used in calculating the RMSD value between the modelled structure and the template.

Molecular Docking

Ligand Generation and Optimization.

ACD-Chemsketch was used in drawing the structure of the ligands taken for binding analysis. Catalyst algorithm in DS was used in ligand preparation with constraint parameters such as tautomer and isomer generation, removal of all the duplicate structures and generation of the 3D structure.

Docking Studies

Docking plays a significant role in predicting binding orientation and affinity of small molecule drug candidates to their known 3D structures [18, 19] of the protein targets. GOLD 4.1 (Genetic Optimization for Ligand Docking) from Cambridge Crystallographic Data center, UK which uses a genetic algorithm for docking ligands into protein binding sites explores the full range of ligand conformational flexibility with partial flexibility of protein. Protein coordinates from the crystal structure of alpha haemolysin (PDB ID: 3M2L), determined at a resolution of 2.10 Å were used to define the active site [20]. All the water molecules present in the protein were removed and hydrogen atoms were added. The active site was defined with a 10 Å radius around the ligand present in the crystal structure. At the end of the computation, the 10 top-scoring conformations of every ligand were saved. Early termination option was applied to pass over the genetic optimization calculation when any five conformations of a particular compound were envisaged within an RMS deviation value of 1.5 Å. The GOLD fitness score is calculated from the contributions of hydrogen bond and van der Waals interactions between the protein and ligand, intramolecular hydrogen bonds and strains of the ligand. Similarly, the docking procedure was performed with the modelled structure of Cysteine protease. The protein–ligand interactions were analysed by silver.

3. Results and Discussion

Homology modelling, preparation of the target protein structure and energy minimization.

The homologue search for the best template for modeling of Cysteine protease (accession number I6V9L0) with 323 amino acids as well as energy minimization of the modelled structure was carried out using DS. CHARMm force field and steepest descent method is applied with 0.001 minimizing RMS gradient and 2000 minimizing steps followed by conjugant gradient method till satisfactory results were obtained for minimization. The energy refinement method gives the best conformation to the modeled structure shown in figure 1. Similar energy refinement process was carried out for the structure of Alpha haemolysin shown in figure 2(PDB ID: 3M2L).

Fig 1: Modelled structure of **Cysteine** protease of BmNPV

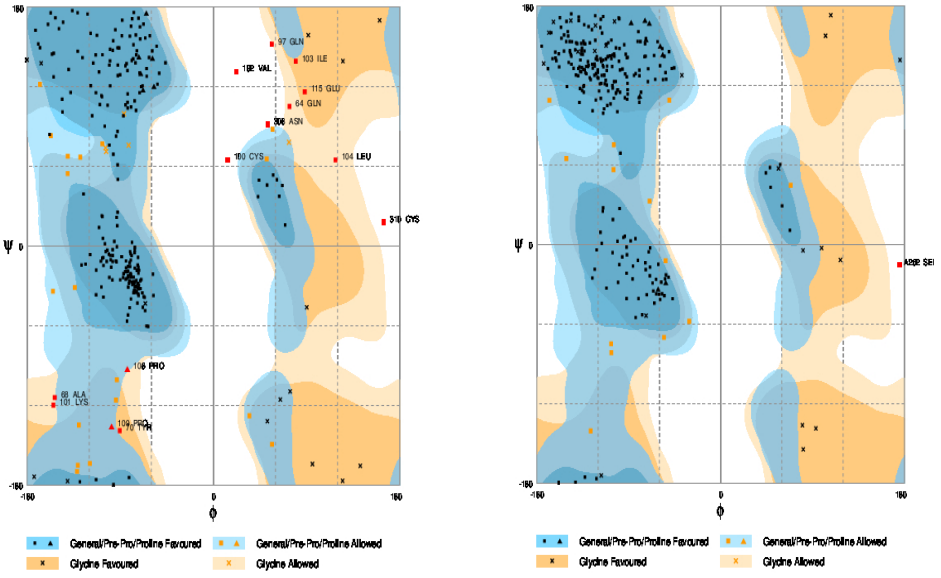
Fig 2: Structure of Alpha Haemolysin (PDB ID: 3M2L) of *Staphylococcus aureus*

Model Validation

To evaluate the quality of the modelled structure of Cysteine protease of BmNPV, the final refined model was analyzed by the RAMPAGE Server, Prosa, and RMSD calculation.

RAMPAGE Server - Ramachandran Plot Analysis

RAMPAGE Server used for validation of protein structure, revealed that the modeled structure Cysteine protease of BmNPV has significant stereochemical quality in Ramachandran plot with favorable region (90.9%), allowed region (6.1%), and disallowed region (3.0 %) respectively. Similarly ramchandran plot analysis of Alpha haemolysin reveals the structure with favorable region (95.2%), allowed region (4.5%), and disallowed region (0.3 %) respectively. The ramchandran plot's and their statistics of the modeled structure Cysteine protease of BmNPV and Alpha haemolysin (PDB ID: 3M2L) of *Staphylococcus aureus* are shown in figure 3 and table 1.



(a) **(b)**

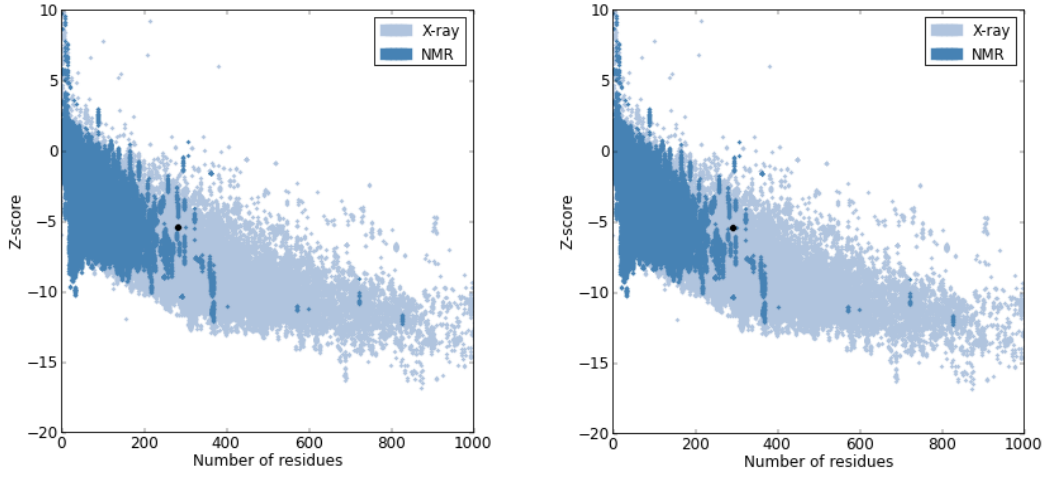
Fig 3: Ramachandran's plot of (a) Cysteine protease of BmNPV and (b) Alpha haemolysin (PDB ID: 3M2L) of *Staphylococcus aureus.*

Table 1: Percentage of residues in the favorable region, allowed region and disallowed regions of the Ramachandran's plot for the homology modeled Cysteine protease of BmNPV and structure of Alpha Haemolysin of *Staphylococcus aureus* (3M2L).

Structure	Favorable region	Allowed region	Disallowed region
Modelled structure of Cysteine protease	90.9%	6.1%	3.0%
Alpha Haemolysin (3M2L)	95.2%	4.5%	0.3%

Prosa.

Quality assessment of the modelled structure was viewed using Prosa program as shown below. The z-score of modelled Cysteine protease was found to be –5.44 and that of alpha haemolysin to be –5.44 which are shown in figure 4.

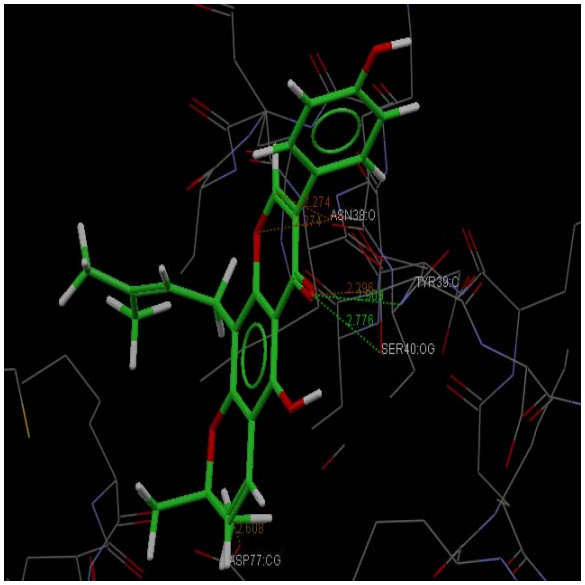


(a) **(b)**

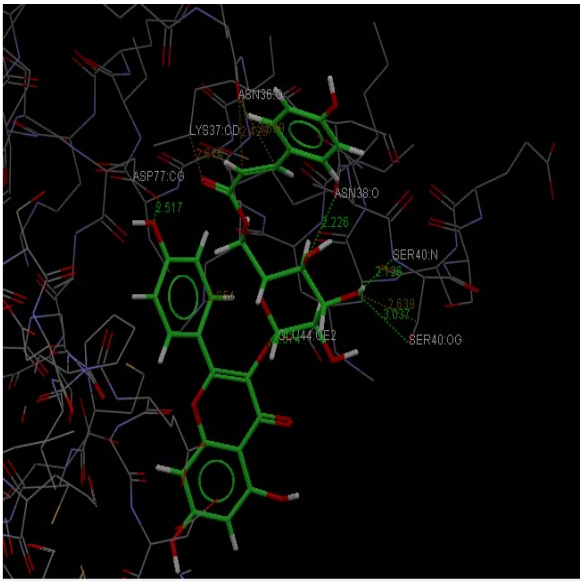
Fig 4: PROSA analysis of (a) Modeled Cysteine protease of BmNPV and (b) Alpha haemolysin (PDB ID: 3M2L) of *Staphylococcus aureus* showing residues at NMR region.

RMSD.
The low RMSD observed between the target protein modeled Cysteine protease of BmNPV and template reflects the presence of strong homology. It was found to be 0.89A.

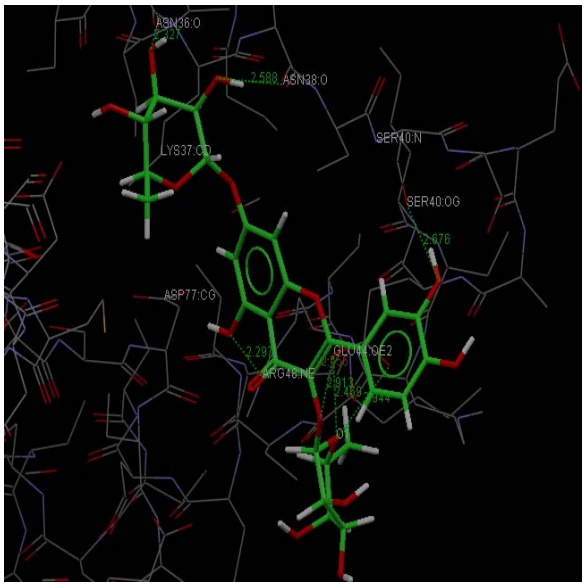
Docking studies
Antimicrobial activity of pure flavonoid derivatives or flavonoid-containing plant extracts has been extensively studied and reviewed [21, 22, 23, 24, 25]. Quercetin-3,7-O-α-l-dirhamnoside and kaempferol are known to be the most common flavonols present in many plants in different glycosidic forms and antimicrobial activity of plant extracts (e.g. Rubus ulmifolius, Combretum erythrophyllum, Morus alba, Trollius chinensis, and propolis) has been attributed to due to presence of this compounds [26, 27, 28, 29, 30, 31].
Docking studies were carried out with the both the proteins (modelled structure of the BmNVP and 3M2L of alpha haemolysin) into the active site with the selected ligands (figure 7) using the GOLD (Genetic Optimization for Ligand Docking) docking program. GOLD fitness score which differentiates molecules on account of their interacting pattern is calculated for all the four molecules. The docking scores of the four flavonoids and various hydrogen-bonding interactions exhibited by them with the key active site residues of modelled structure of the BmNVP are shown in the figure 5 and table 2. The docking scores of the four flavonoids and various hydrogen-bonding interactions exhibited by them with the key active site residues of alpha haemolysin are shown in the figure 6 and table 3. Our results demonstrate that scandenone and quercetin-3,7-O-α-l-dirhamnoside possess antibacterial as well as antiviral activity.



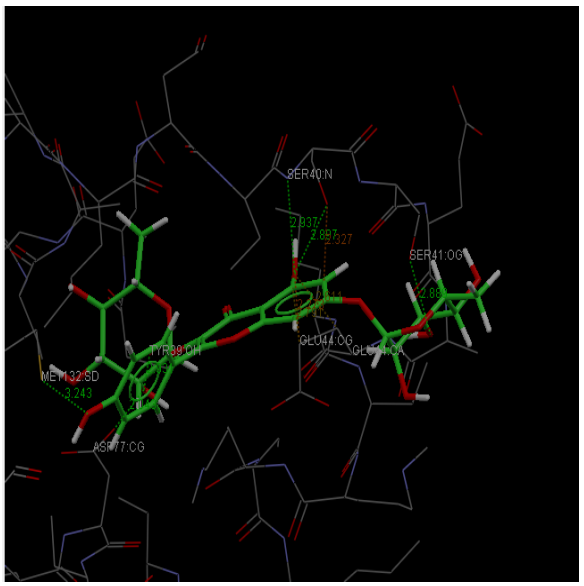
(a) scandenone



(b) tiliroside

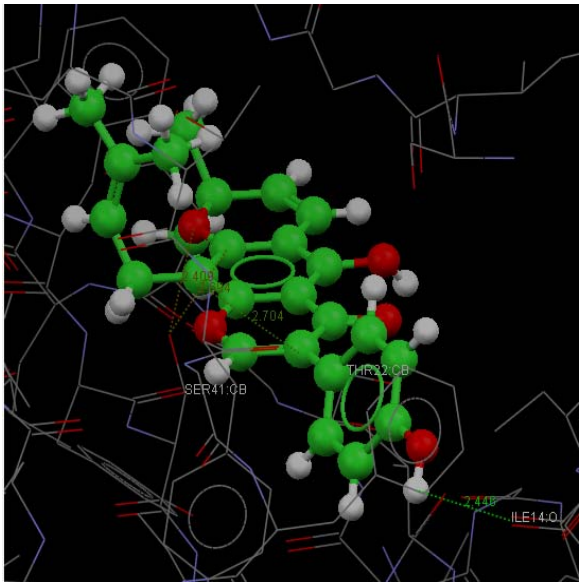


(c) Quercetin-3,7-O-α-l-dirhamnoside

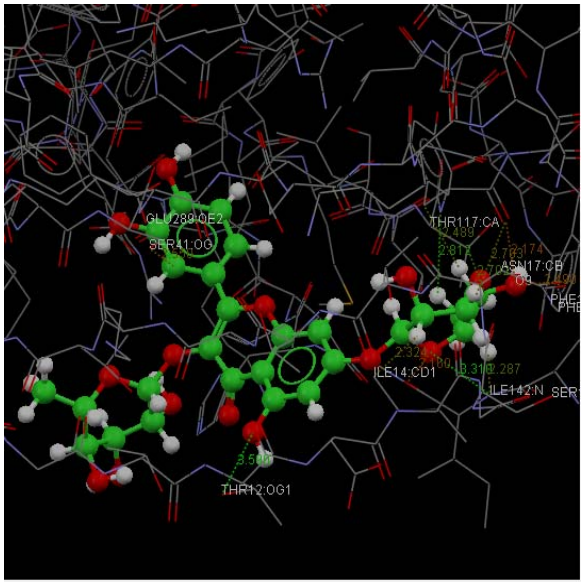


(d) Kampeferol

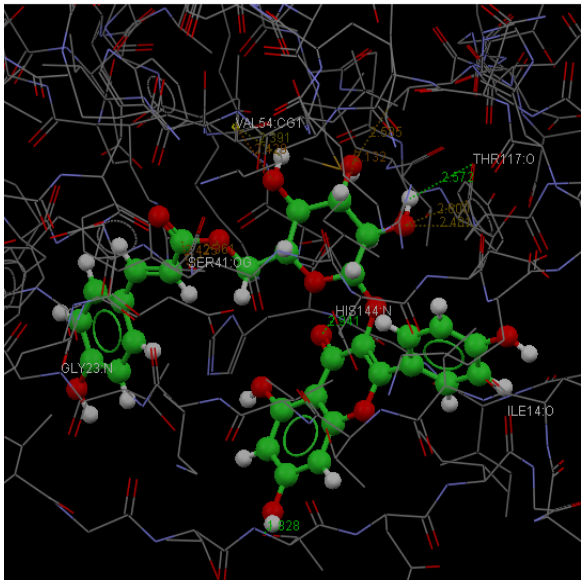
Fig 5: H-bonding interactions of the modeled viral cathepsin of BmNPV with a) scandenone (b) tiliroside (c) Quercetin-3,7-O-α-l-dirhamnoside (d) Kampeferol.



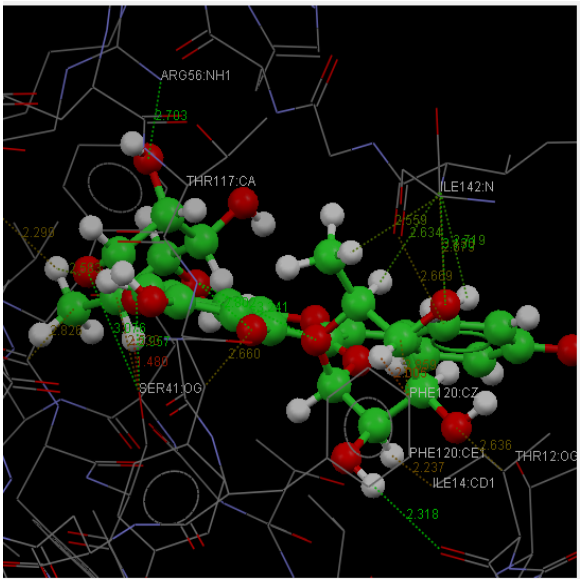
(a) scandenone



(b) tiliroside



(c) Quercetin-3,7-O-α-l-dirhamnoside



(d) Kampeferol

Fig 6: H-bonding interactions of the Alpha Haemolysin (PDB ID: 3M2L) of *Staphylococcus aureus* with a) scandenone (b) tiliroside (c) Quercetin-3,7-O-α-l-dirhamnoside (d) Kampeferol.

Table 2: Docking scores of the four flavonoids with modeled Cysteine protease

Name of the comp	Fitness value	S(hb_ext)	S(vdw_ext)	S(hb_int	S(int)
Scandenone	34.70	5..42	26.94	0.0	-7.76
Tiliroside	28.21	6.16	37.39	0.0	-29.37
Quercetin-3,7-O-α-l-dirhamnoside	37.15	2.49	31.84	0.0	-9.12
Kampeferol	28.39	6.0	30.36	0.0	-19.36

Table 3: Docking scores of the four flavonoids with Alpha hamelysin of *Staphylococcus aureus*

Name of the comp	Fitness value	S(hb_ext)	S(vdw_ext)	S(hb_int	S(int)
Scandenone	52.24	0.37	43.88	0.0	-8.46
Tiliroside	50.63	3.24	50.05	0.0	-21.44
Quercetin-3,7-O-α-l-dirhamnoside	49.40	5.46	46.33	0.0	-19.76
Kampeferol	40.82	1.24	43.52	0.0	-20.26

4. Conclusions

Sericulture is cultivation of silkworms which produces silk, queen of textile and the naturally produced animal fibre. Due to various biological, chemical, physical, nutritional and environmental factors, silkworms are affected by a number of diseases there by effecting silk production. Among various pathogenic microbes causing disease in silkworm, virus *Bombyx mori nuclear polyhedrosis virus* (BmNPV) and bacteria *Staphylococcus aureus* were found to be responsible for silkworm mortality. In the present study, Flavonoids which have been reported to possess broad spectrum of antimicrobial activity against a wide range of pathogens have been evaluated using insilico techniques. Four selected flavonoids that include scandenone, tiliroside, quercetin-3,7-O-α-l-dirhamnoside, and kaempferol were elucidated for antiviral as well as antibacterial activity. Docking studies revealed that scandenone and quercetin-3,7-O-α-l-dirhamnoside possess antibacterial as well as antiviral activity. Future studies would be focused on validation of these results by wet lab studies.

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