

Research Article

Predicting the Potential of Black Seed Bioactive Compounds against Potato Virus X Using *In Silico* Molecular Docking

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Abstract | This study presents a comprehensive analysis of the physicochemical, pharmacokinetic, and molecular docking properties of black seed (*Nigella sativa*) bioactive compounds-Nigellicine, Carvacrol, Nigellidine, and Thymoquinone-compared to the antiviral drug Ribavirin, using SwissADMET predictions and molecular docking simulations. The physicochemical profiles revealed that all black seed compounds are drug-like, with molecular weights under 500 g/mol, low molecular flexibility, and adherence to Lipinski's rule. Black seed compounds exhibit high gastrointestinal absorption and positive blood-brain barrier (BBB) permeability, suggesting better bioavailability and potential CNS activity than Ribavirin. Notably, Carvacrol and Nigellidine inhibit key cytochrome P450 enzymes (CYP1A2 and CYP2D6), which could lead to drug-drug interactions. Molecular docking results demonstrated that Nigellidine showed the highest binding affinity and docking score against both PVX replicase (-8.9 kcal/mol) and PVX coat protein (-8.1 kcal/mol), outperforming Ribavirin. Nigellicine also exhibited promising docking scores, comparable to Ribavirin. In contrast, Carvacrol and Thymoquinone showed weaker interactions. These findings suggest that Nigellidine and Nigellicine have superior potential as antiviral agents, with stronger and more diverse interactions than Ribavirin, especially in targeting PVX proteins. This study highlights the therapeutic potential of black seed compounds for antiviral drug development and provides a foundation for future experimental validation.

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Introduction

Potato virus X (PVX) is one of the most important pathogens affecting potato crops globally

(Verchot, 2022). As a member of the Potexvirus genus, PVX is primarily transmitted through mechanical means, such as tools and human activity, as well as *via* seeds and vectors (Verchot, 2022). The virus causes

a variety of symptoms, including mosaic patterns, stunted growth, leaf deformation, and ultimately a reduction in yield and quality of the potato tubers (Pourrahim *et al.*, 2007). This has a severe economic impact on global potato production, particularly in regions where potato is a staple crop (Denaro *et al.*, 2020; Verchot, 2022). PVX not only affects the potato industry but also serves as a model organism for studying plant viral diseases, making its control critical for agricultural research and sustainable food production (Zhao and Cafilisch, 2015).

Nigella sativa, commonly known as black seed, is a medicinal plant with a long history of use in traditional medicine across various cultures (Dabeer *et al.*, 2022). Its seeds contain a wide range of bioactive compounds, including alkaloids, flavonoids, terpenes, and essential oils, such as thymoquinone, thymohydroquinone, and carvacrol. These compounds are renowned for their therapeutic properties, which include antioxidant, anti-inflammatory, antimicrobial, and antiviral activities (Gaurav *et al.*, 2022; Gholamnezhad *et al.*, 2016). Research has demonstrated that black seed oil, particularly thymoquinone, can inhibit the replication of several viruses, including some human viruses (Abbas *et al.*, 2024). Moreover, thymoquinone has been found to enhance immune responses and exhibit antitumor and anticancer properties, making black seed a promising candidate for combating various infectious diseases (Darakhshan *et al.*, 2015).

Current methods of controlling PVX include conventional chemical treatments, which often have limited efficacy and can harm the environment (Karan *et al.*, 2021). Additionally, genetically modified crops that are resistant to PVX have been developed, but they face regulatory and public acceptance challenges (Reddy *et al.*, 2009). Therefore, there is a growing interest in exploring natural compounds, particularly plant-derived bioactive substances, as alternative antiviral agents that can control PVX infection effectively and sustainably.

Despite the vast range of biological activities attributed to black seed, limited research has focused on its potential antiviral effects against plant viruses (Basurra *et al.*, 2021). As a result, the exploration of black seed bioactive compounds as potential antiviral agents against PVX presents a novel avenue for plant disease management. In particular, molecular docking studies, which utilize computational techniques to

predict the interactions between ligands and target proteins, offer a valuable tool for identifying potential bioactive compounds from black seed (Ferreira *et al.*, 2015; Jakhar *et al.*, 2020) that can effectively inhibit PVX replication.

Molecular docking is an *in silico* technique that simulates the binding interactions between small molecules (ligands) and macromolecules (such as proteins or nucleic acids) at the atomic level. This technique provides insights into the binding affinity, orientation, and specificity of a ligand to its target protein (Morris and Lim-Wilby 2008). It has been widely used in drug discovery (Pinzi and Rastelli, 2019), especially when considering plant-derived compounds (Bekhit and Bekhit, 2014; Sangeetha *et al.*, 2020). By predicting the interaction between bioactive compounds and viral proteins, docking studies can help prioritize compounds for experimental validation, saving time and resources in the process of drug or pesticide development (Tallei *et al.*, 2020).

In the context of PVX, molecular docking can be employed to predict how bioactive compounds from black seed interact with key viral proteins, such as the viral coat protein (CP), which is essential for the virus's infectivity, and the replicase complex, which is involved in viral replication. The successful binding of black seed-derived compounds to these viral targets could inhibit PVX replication and reduce the severity of the infection. Additionally, molecular docking studies can explore the binding mechanisms of compounds like thymoquinone, which is thought to interfere with the viral life cycle by disrupting protein function or viral replication (Hafez *et al.*, 2024). By investigating a range of bioactive compounds from black seed, researchers can identify those with the highest potential to combat PVX.

Therefore, the objectives of this study were to evaluate the potential of black seed bioactive compounds against PVX using an *in silico* molecular docking approach. The specific objectives of the study are: To identify and extract bioactive compounds from *Nigella sativa* that have demonstrated antiviral properties in previous research. To perform molecular docking simulations to predict the binding interactions of these compounds with key viral proteins of PVX, including the viral coat protein and replicase enzymes. To assess the binding affinity, stability, and specificity of the

interactions between these compounds and the viral proteins. To compare the effectiveness of black seed bioactive compounds with known antiviral agents, providing a basis for future experimental studies.

Materials and Methods

Selection of target proteins

The key proteins of PVX, replicase (Rep) (Accession: YP_002332929) and coat protein (CP) (Accession: YP_002332933), were identified as potential docking targets. Their protein sequences were retrieved from the NCBI database (<https://www.ncbi.nlm.nih.gov>) in FASTA format. To obtain their 3D structures in PDB format, a search was conducted on the Protein Data Bank (PDB) (<https://www.rcsb.org/>). The 3D models of the target proteins were generated using homology modeling tools such as SWISS-MODEL or Phyre2, based on sequence similarity to known structures. The modeled structures were validated for structural accuracy using tools like Ramachandran plot analysis. In this study, protein structures were computationally predicted through a homology-based approach, utilizing known protein structures with high sequence identity to the target PVX proteins as templates. The sequences of the target proteins were aligned with the templates to generate the 3D models.

Selection of bioactive compounds from black seed

Four major bioactive compounds known for their antiviral properties-Thymoquinone, Nigellidine, Carvacrol, and Nigellicine-were selected from literature. Their chemical structures were retrieved in 2D SDF format from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>).

Virtual screening of bioactive compounds

A small compound library containing the selected bioactive compounds was prepared. Virtual screening was performed using PyRx software (<https://sourceforge.net/projects/pyrx/>), which integrates energy minimization and docking through the AutoDock Vina plugin. AutoDock Vina and CB-Dock were selected for molecular docking due to their established performance, versatility, and reliability in docking studies of small molecules and protein-ligand interactions: AutoDock Vina: Known for its speed, accuracy, and ease of use, AutoDock Vina is a preferred tool for docking simulations. It offers a robust scoring function and has been validated in numerous

studies, particularly for predicting binding affinities and poses of ligand-protein complexes. Its efficiency and ability to handle large numbers of docking poses made it suitable for this study. CB-Dock: Specifically designed for docking small molecules to the cavities of macromolecules, CB-Dock provides accurate predictions of protein-ligand interactions, especially in flexible and larger binding sites, making it highly suitable for the targets in this study. The compounds were screened against the replicase and coat protein of PVX to prioritize those with lower binding energy values, indicative of stronger binding potential. The 2D structures were converted to 3D and optimized for energy minimization using Open Babel, Chem3D. Additionally, Lipinski's rule of five was applied using tools like SwissADME to assess their drug-likeness and potential bioavailability.

Molecular docking

The following steps were undertaken to perform detailed docking simulations. The target proteins were preprocessed by: Removing water molecules and irrelevant ligands using PyMOL. Adding hydrogen atoms and assigning Kollman or Gasteiger charges. Energy minimization of ligands was carried out using Open Babel or Chem3D. This ensured geometrical stability for accurate docking. Molecular docking was performed using CB-Dock (CB-Dock) and AutoDock Vina. The docking scores (binding affinities in kcal/mol) were recorded for each protein-ligand interaction. CB-Dock also facilitated automatic identification of binding sites, enhancing docking accuracy.

Visualization and analysis

Docking results were analyzed and visualized using Discovery Studio 2022 (<https://10.0.142.116/Pharmaceutical-Sciences.543>) to identify critical interactions such as hydrogen bonds, hydrophobic contacts, and ionic interactions.

Results

Data in Table 1 present a comparative analysis of the physicochemical and pharmacokinetic properties of several bioactive components of black seed (*Nigella sativa*), including Nigellidine, Carvacrol, Nigellidine, and Thymoquinone compared to Ribavirin (a well-known antiviral drug) using SwissADMET predictions.

Table 1: *Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of black seed components compared to ribavirin.*

Swiss ADMET	Control	Black seed components			
	Ribavirin	Nigellicine	Carvacrol	Nigellidine	Thymoquinone
Molecular weight) (g/ mole)	244.20	246.26	150.22	294.35	164.20
No. rotatable bonds	3	1	1	1	1
No. H-bond acceptors	7	3	1	2	2
Num. H-bond donors	4	1	1	1	0
GI absorption	Low	High	High	High	High
BBB permeant	No	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	Yes	Yes	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	Yes	No
CYP3A4 inhibitor	No	No	No	No	No
Lipinski	Yes	Yes	Yes	Yes	Yes

Table 2: *Virtual screening and docking scores and results of ligands against Potato virus X (PVX)-replicase.*

Ligands	Virtual screening			Docking scores
	Binding affinity	rmsd /ub	rmsd/lb	
Potato virus X -Replicase_Carcacrol	-5.8	0	0	-6.0
Potato virus X -Replicase_Nigellicine	-7.3	0	0	-7.2
Potato virus X -Replicase_Nigellidine	-8.9	0	0	-8.4
Potato virus X -Replicase_Thymoquinone	-5.5	0	0	-6.1
Potato virus X -Replicase_Ribavirin (control)	-7.2	0	0	-7.3

Where; rmsd /ub: Root Mean Square Deviation/unit of biology. rmsd/lb: Root Mean Square Deviation/Lower Bound.

The experimental results showed some general observations could be considered. All black seed components have molecular weights within the range suitable for drug-likeness (<500 g/mol), comparable to Ribavirin. The components exhibit low molecular flexibility, as evidenced by their low number of rotatable bonds (1–3). Black seed components have fewer hydrogen bond donors and acceptors than Ribavirin, which might influence their solubility and interaction with biological targets. All black seed components demonstrate high Gastrointestinal (GI) absorption, in contrast to the low absorption of Ribavirin, suggesting better oral bioavailability potential. Black seed components show positive predictions for BBB permeability, indicating potential central nervous system (CNS) activity, whereas Ribavirin does not cross the BBB. Among the black seed components, Carvacrol and Nigellidine inhibit CYP1A2, while Nigellidine is a CYP2D6 inhibitor. This indicates possible drug-drug interactions involving these enzymes. Ribavirin, on the other hand, does not inhibit any cytochrome P450 enzymes. Finally, all the

compounds comply with Lipinski's rule, supporting their drug-likeness.

Results suggested that black seed components, particularly Thymoquinone, may have significant pharmacokinetic advantages over Ribavirin, including better GI absorption, CNS penetration, and favorable drug-likeness properties. These findings align with the traditional use of *N. sativa* as a therapeutic agent and warrant further exploration of its components as potential drug candidates.

The binding affinities and docking scores of four bioactive compounds (Carvacrol, Nigellicine, Nigellidine, and Thymoquinone) are presented in Table 2 in the presence of the standard antiviral drug Ribavirin against PVX-Replicase. Docking scores represent the ligand-receptor binding efficiency, where lower scores (more negative values) indicate stronger binding affinity. Among the tested compounds, Nigellidine showed the highest binding affinity (-8.9 kcal/mol) and the most favorable docking score (-8.4

kcal/mol), suggesting strong potential as an inhibitor of PVX-Replicase.

This may be attributed to the structural compatibility and higher molecular interaction energies between Nigellidine and the active site of PVX-Replicase. Nigellidine (-7.3 kcal/mol) exhibited comparable docking results to Ribavirin (-7.2 kcal/mol), indicating its promise as an alternative antiviral candidate. However, Carvacrol and Thymoquinone demonstrated weaker binding affinities (-5.8 kcal/mol and -5.5 kcal/mol, respectively), suggesting lower inhibitory potential relative to the other ligands.

The control drug, Ribavirin, serves as a benchmark, with binding affinity (-7.2 kcal/mol) and docking scores (-7.3 kcal/mol) closely mirroring those of Nigellidine. This validates the docking methodology, as Ribavirin is a known antiviral agent with activity against replicase enzymes.

The docking scores and binding affinities of selected ligands of black seed under investigation that presented in Table 3 were compared to the control drug Ribavirin against PVX-coat protein. The results highlight variations in binding efficiency and molecular interaction between the ligands and the protein target.

Nigellidine demonstrated the strongest binding affinity (-8.1 kcal/mol) and the most favorable docking score (-8.3 kcal/mol) among the tested compounds, indicating its potential as a potent inhibitor of PVX-coat protein. Its performance surpasses that of the control drug Ribavirin (-7.7 kcal/mol binding affinity and -7.4 kcal/mol docking score), further underscoring its potential for therapeutic application. Nigellidine also exhibited a relatively strong interaction with the PVX-Coat Protein, with a binding affinity of -7 kcal/mol and a docking score of -8 kcal/mol. These

results suggest that both Nigellidine and Nigellidine could act as promising leads for further antiviral drug development targeting PVX-Coat Protein.

On the other hand, Carvacrol (-6.3 kcal/mol) and Thymoquinone (-6 kcal/mol) showed weaker binding affinities, indicating less favorable interactions with the protein target. This aligns with the trend observed in the previous table for their interaction with PVX-Replicase. These results suggest that these compounds may not be optimal candidates for targeting PVX-Coat Protein.

The control drug Ribavirin demonstrated reliable binding with PVX-Coat Protein (-7.7 kcal/mol binding affinity), validating the docking procedure and further highlighting Nigellidine's superior binding affinity. This is consistent with its role as a benchmark antiviral agent.

The results in Table 4, illustrated in Figures 1-5, provide a detailed analysis of the interaction profile between black seed compounds and the PVX replicase protein. The table lists five ligands: Ribavirin (as a control) and four black seed compounds—Carvacrol, Nigellidine, Nigellidine, and Thymoquinone highlighting the specific amino acids involved, binding sites, and bond types. Each ligand interacts with distinct binding sites on the protein (e.g., LYS A:805, GLN A:832), suggesting these sites are critical for recognition and binding. The various bond types (e.g., Pi-Alkyl, Pi-Cation, Alkyl, and Hydrogen bonds) indicate diverse interaction mechanisms, contributing to the stability and binding affinity of the ligand-protein complex.

Ribavirin as control was selected because it is a well-established antiviral drug known for its broad-spectrum antiviral activity, including efficacy against plant viruses. It has been widely used in research involving various viral pathogens, including PVX

Table 3: Docking scores and virtual screening results of ligands against Potato virus X (PVX)-coat protein.

Ligand	Virtual screening			Docking scores
	Binding affinity	rmsd/ub	rmsd/lb	
Potato virus X -Coat-Protein_Carcacrol	-6.3	0	0	-6.3
Potato virus X -Coat-Protein_Nigellidine	-7	0	0	-8.0
Potato virus X -Coat-Protein_Nigellidine	-8.1	0	0	-8.3
Potato virus X -Coat-Protein_Thymoquinone	-6	0	0	-6.6
Potato virus X -Coat-Protein_Ribavirin (control)	-7.7	0	0	-7.4

Where; rmsd /ub: Root Mean Square Deviation/unit of biology. rmsd/lb: Root Mean Square Deviation/Lower Bound.

Table 4: Interaction profile of black seed compounds targeting Potato virus X (PVX) replicase protein: amino acids, bond types and binding sites.

Ligands	Amino acids	Cites	Type of bonds
Black seed ligands			
Carvacrol (4 Bonds)	LYS	A:805	Alkyl
	ASP	A:802	Pi-Cation
	GLN	A:832	Pi-Donor Hydrogen
	LYS	A:741	Pi-Alkyl
Nigellicine (7 Bonds)	TRP	A:771	Pi-Alkyl
	SER	A:742	Pi-Donor Hydrogen
	ARG	A:867	Conventional Hydrogen
	GLY	A:740	Conventional Hydrogen
	LYS	A:741	Conventional Hydrogen
	HIS	A:743	Carbon Hydrogen
	THR	A:929	Alkyl
Nigellidine (8 Bonds)	ARG	A:867	Pi-Cation
	TRP	A:771	Pi-Alkyl
	SER	A:742	Pi-Donor Hydrogen
	GLN	A:832	Conventional Hydrogen
	GLY	A:735	Conventional Hydrogen
	LYS	A:741	Pi-Alkyl
	GLY	A:736	Pi-Donor Hydrogen
	HIS	A:743	Pi-Alkyl
Thymo-quinone (3 Bonds)	LYS	A:805	Alkyl
	LYS	A:741	Pi-Cation
	GLN	A:832	Pi-Donor Hydrogen
Control			
Ribavirin (3 Bonds)	LYS	A:805	Pi-Alkyl
	LYS	A:741	Pi-Cation
	GLN	A:832	Pi-Donor Hydrogen

and other related plant viruses (Lerch, 1977, 1987; Danci et al., 2009; Karjadi and Gunaeni, 2022). As a control, Ribavirin forms 3 bonds with LYS (A:805), LYS (A:741), and GLN (A:832) through Pi-Alkyl, Pi-Cation, and Pi-Donor Hydrogen bonds, respectively. Ribavirin serves as a baseline for comparing the efficacy of the black seed compounds.

Carvacrol interacts with LYS (A:805), ASP (A:802), GLN (A:832), and LYS (A:741), forming a mix of Alkyl, Pi-Cation, Pi-Donor Hydrogen, and Pi-Alkyl bonds. These interactions suggest that Carvacrol engages the protein through both hydrophobic and electrostatic forces, potentially contributing to its antiviral activity.

Nigellicine, a more complex ligand with 7 bonds, forms a range of interactions, including Pi-Alkyl (TRP A:771), Pi-Donor Hydrogen (SER A:742), Conventional Hydrogen (ARG A:867, GLY A:740, LYS A:741), and Alkyl (THR A:929). The greater number of interactions suggests that Nigellicine may have a more extensive and stable binding profile, potentially leading to stronger inhibition of PVX replicase.

Nigellidine, with 8 bonds, forms interactions with ARG (A:867), TRP (A:771), SER (A:742), GLN (A:832), GLY (A:735, A:736), LYS (A:741), and HIS (A:743). The increased number of interactions, including Pi-Cation and Pi-Alkyl bonds, suggests a highly stable and potent binding affinity with the PVX replicase protein.

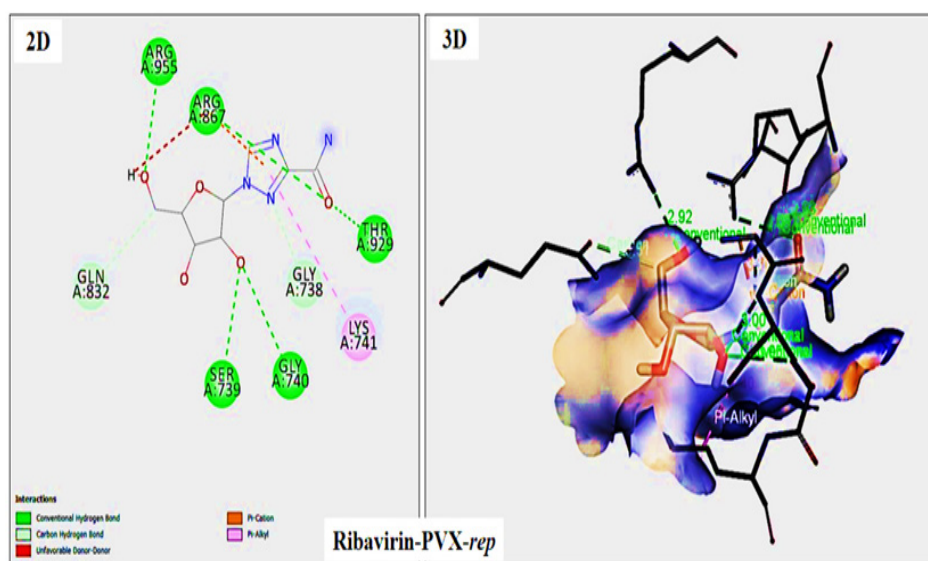


Figure 1: 2D and 3D structural analysis of Ribavirin (Control Ligand) binding to the PVX replicase protein: insights into molecular interactions.

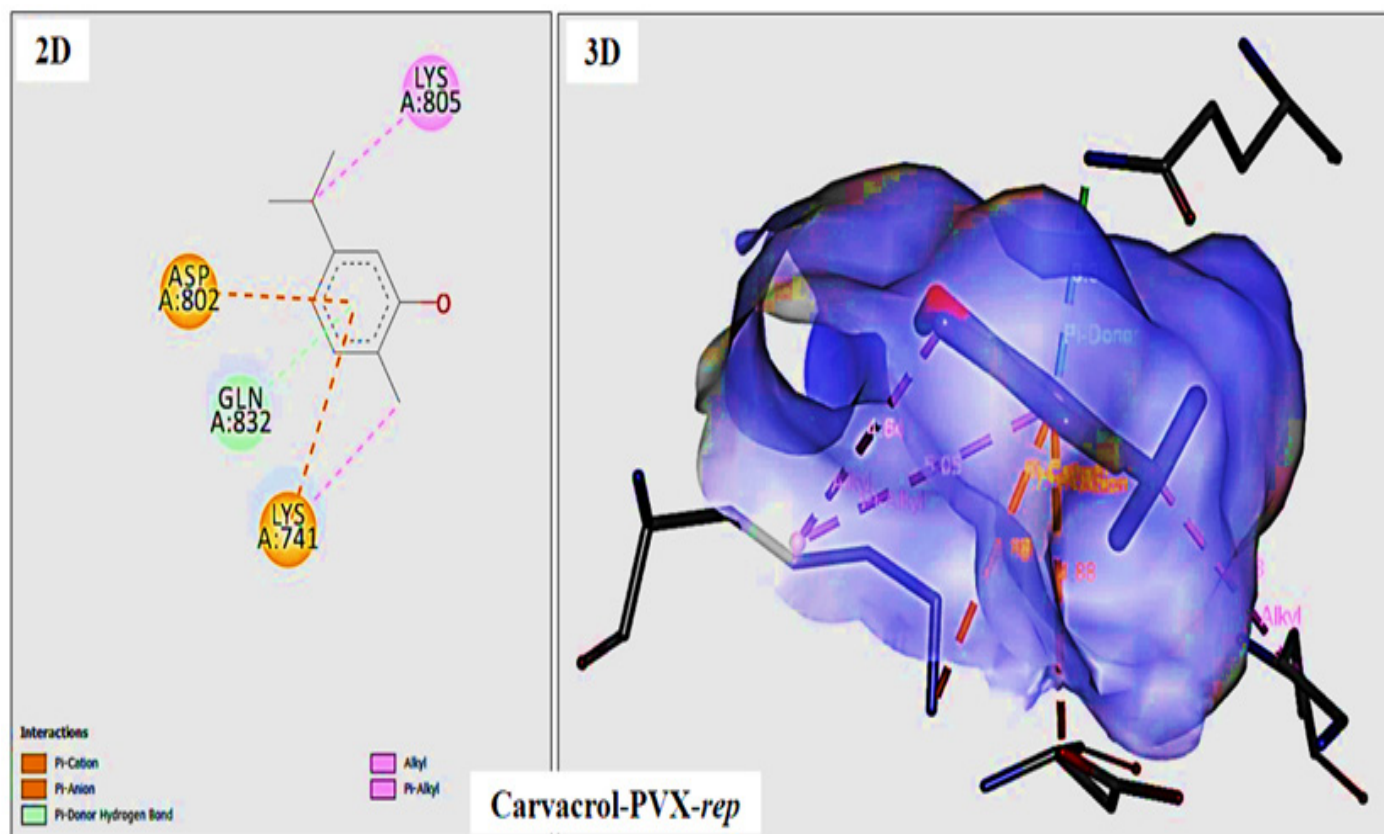


Figure 2: 2D and 3D structural analysis of Carvacrol ligand binding to the PVX replicase protein: insights into molecular interactions.

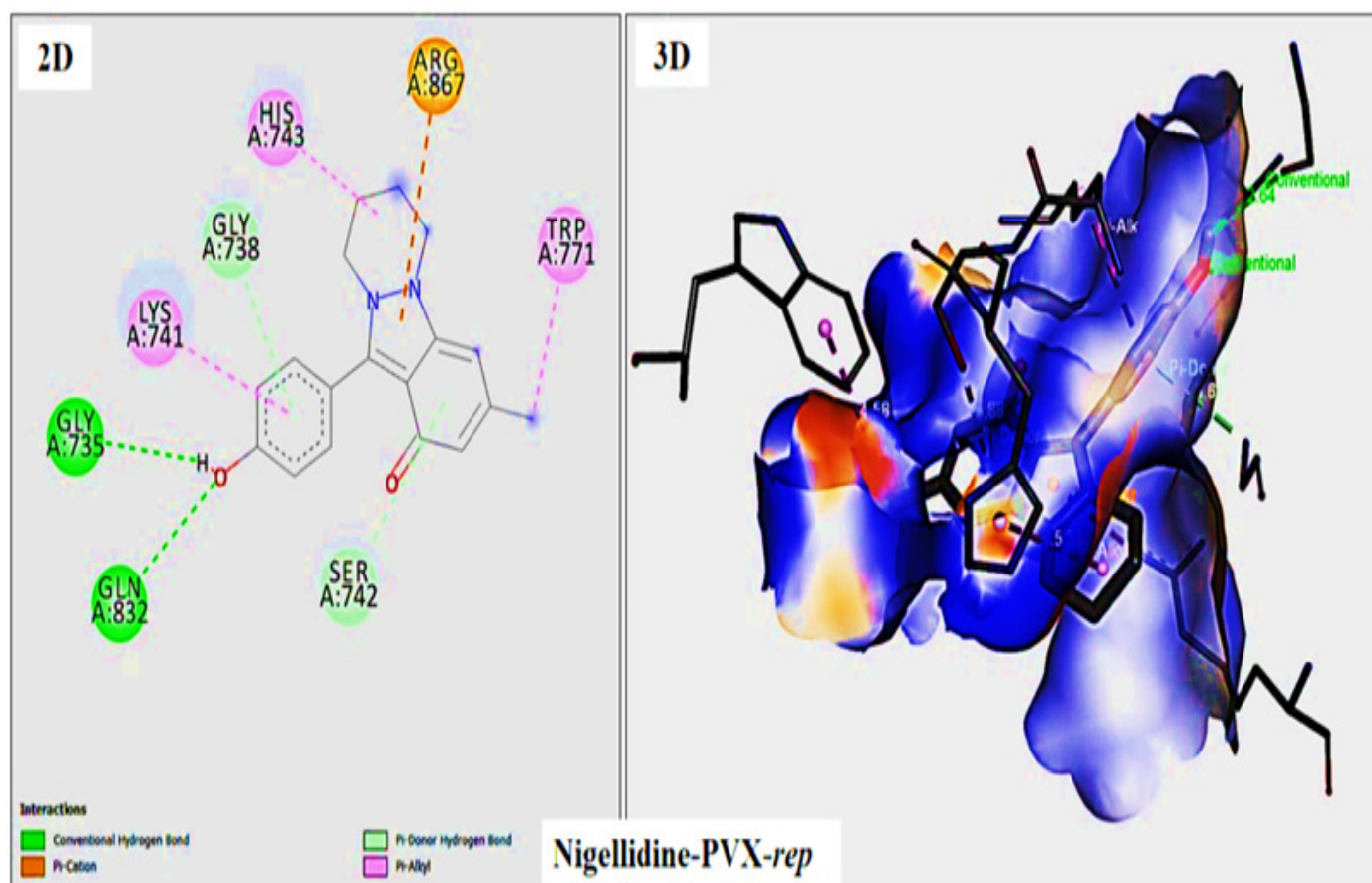


Figure 3: 2D and 3D structural analysis of Nigellidine ligand binding to the PVX replicase protein: insights into molecular interactions.

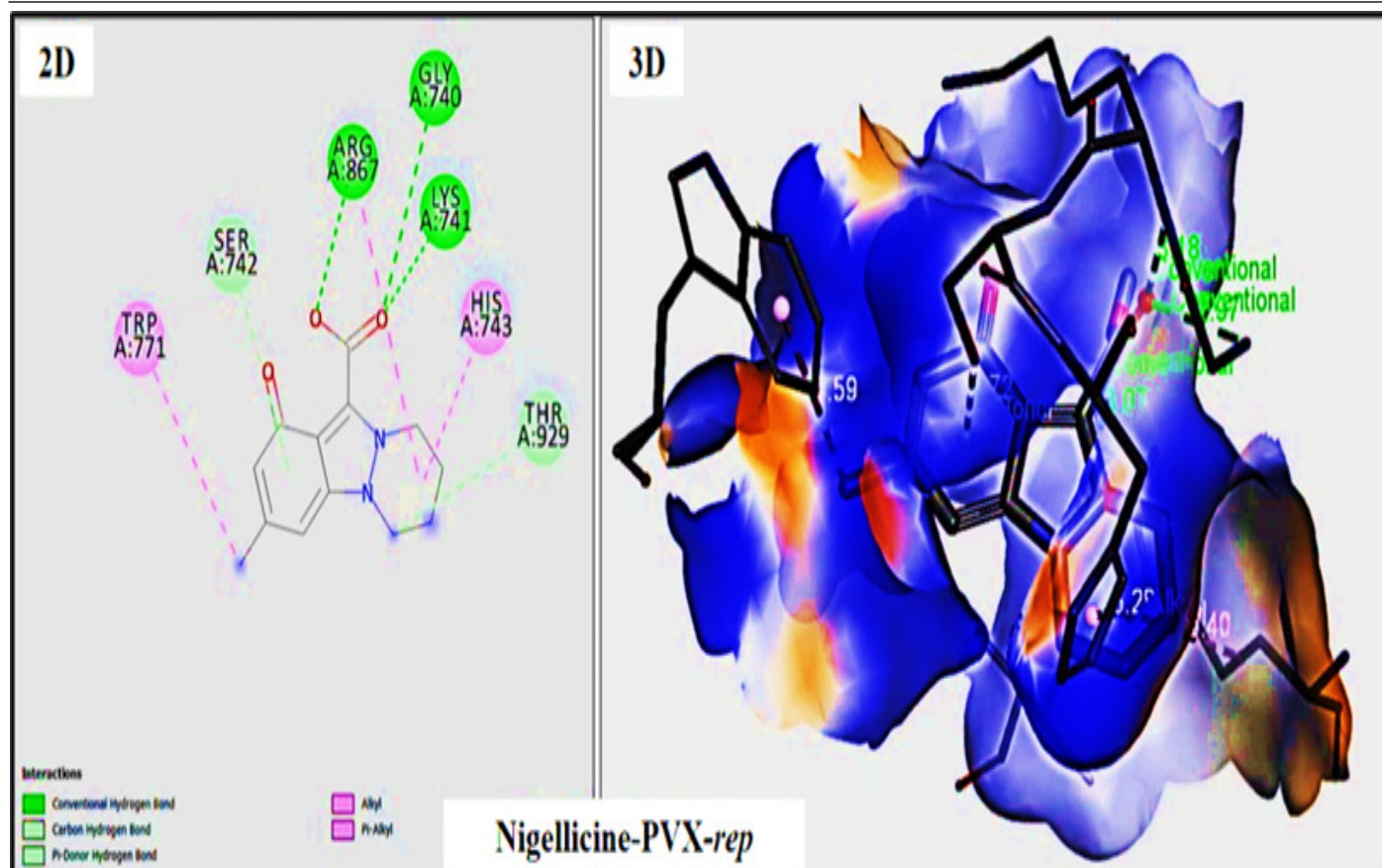


Figure 4: 2D and 3D structural analysis of Nigellidine ligand binding to the PVX replicase protein: insights into molecular interactions.

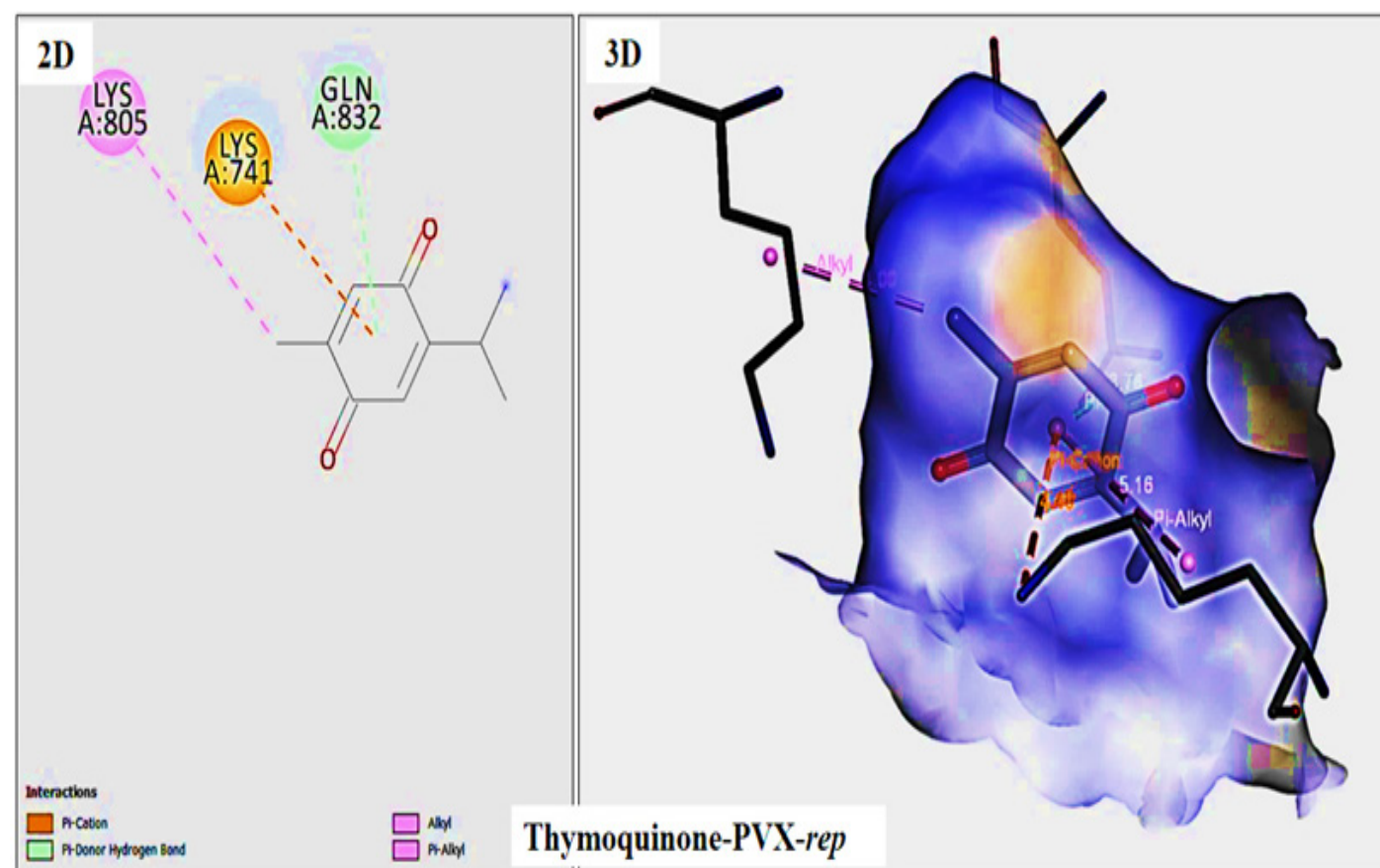


Figure 5: 2D and 3D structural analysis of Thymoquinone ligand binding to the PVX replicase protein: insights into molecular interactions.

Thymoquinone forms 3 bonds with LYS (A:805), LYS (A:741), and GLN (A:832), involving Alkyl, Pi-Cation, and Pi-Donor Hydrogen bonds. Its interaction profile is similar to Ribavirin but with a different bond pattern, suggesting distinct binding dynamics.

Overall, several ligands (e.g., Carvacrol, Nigellidine, Thymoquinone) interact with LYS (A:805) and LYS (A:741), highlighting these sites as crucial for the binding of black seed compounds. The ligands interact with the protein through a variety of bond types, indicating a complex binding mechanism. Pi-Alkyl and Pi-Cation interactions likely reflect hydrophobic and electrostatic binding, while conventional hydrogen and Pi-Donor Hydrogen bonds contribute to binding specificity and stability. Black seed compounds, particularly Nigellidine and Nigellicine, exhibit more interactions than Ribavirin, suggesting stronger and more diverse binding potential, which could lead to more effective inhibition of PVX replicase.

In summary, the table highlights the interaction profiles of black seed compounds with PVX replicase, providing valuable insights into the mechanisms by which these compounds may inhibit viral replication. This information could be crucial for developing more effective antiviral drugs targeting PVX or similar viruses.

Data in Table 5 and Figures 6-10 detailed the interaction profiles of black seed compounds and Ribavirin (as a control) with the PVX coat protein. The interactions are summarized in terms of amino acids, binding sites, and bond types.

Ribavirin (Control) forms 8 bonds with the PVX coat protein, including Conventional Hydrogen (THR B:227, A:223, A:205), Amide-Pi Stacked (ASN A:208), and Van der Waals (ASP A:209), suggesting a mixed hydrophilic binding mechanism.

Carvacrol forms 3 bonds, primarily hydrophobic (Alkyl and Pi-Sigma), indicating binding to non-polar regions of the protein. Nigellidine, with 11 bonds, exhibits a complex binding profile, including Conventional Hydrogen, Alkyl, Pi-Sigma, and Pi-Alkyl interactions. This diversity suggests a strong, versatile binding affinity with both hydrophobic and electrostatic regions of the protein.

Nigellidine forms 7 bonds, including Pi-Sulfur (MET D:128) in addition to Alkyl and Pi-Alkyl interactions, indicating stronger and more stable binding through both hydrophobic and electrostatic interactions. Thymoquinone also forms 7 bonds, with a mix of hydrophobic (Pi-Alkyl, Alkyl) and hydrophilic (Conventional Hydrogen, Pi-Sigma) interactions, suggesting a balanced binding mechanism.

Table 5: Interaction profile of black seed compounds targeting potato virus X (PVX) coat protein: amino acids, bond types and binding sites.

Ligands	Amino acids	Cites	Type of bonds
Black seed ligands			
Carvacrol (3 Bonds)	LYS	D:199	Alkyl
	ALA	D:196	Pi-Sigma
	ALA	D:195	Alkyl
Nigellidine (11 Bonds)	ASN	C:166	Conventional Hydrogen
	PHE	C:165	Conventional Hydrogen
	PHE	C:164	Conventional Hydrogen
	ARG	C:124	Carbon Hydrogen
	LYS	C:199	Alkyl
Nigellidine (7 Bonds)	ILE	C:200	Alkyl
	ASP	C:163	Pi-Sigma
	ALA	C:195	Amide Pi Stacked
	ALA	C:192	Pi-Alkyl
	ALA	C:196	Pi-Alkyl
	PHE	C:153	Alkyls
	LYS	D:199	Alkyl
	ILE	D:200	Alkyl
	PHE	D:153	Pi-Alkyl
	ALA	D:192	Pi-Alkyl
Thymoquinone (7 Bonds)	ALA	D:195	Amide Pi Stacked
	ALA	D:196	Pi-Sigma
	MET	D:128	Pi-Sulfur
	ALA	D:192	Pi-Alkyl
	PHE	D:165	Conventional Hydrogen
	ASN	D:166	Conventional Hydrogen
	ALA	D:196	Pi-Sigma
	ASP	D:163	Pi-Sigma
Control	PHE	D:153	Pi-Alkyl
	ALA	D:195	Alkyl
	THR	B:227	Conventional Hydrogen
	THR	A:223	Conventional Hydrogen
	ALA	A:205	Conventional Hydrogen
	SER	A:207	Conventional Hydrogen
	THR	A:225	Conventional Hydrogen
Ribavirin (8 Bonds)	ASN	A:208	Amide-Pi Stacked
	THR	B:225	Carbon Hydrogen
	ASP	A:209	Van der Waals

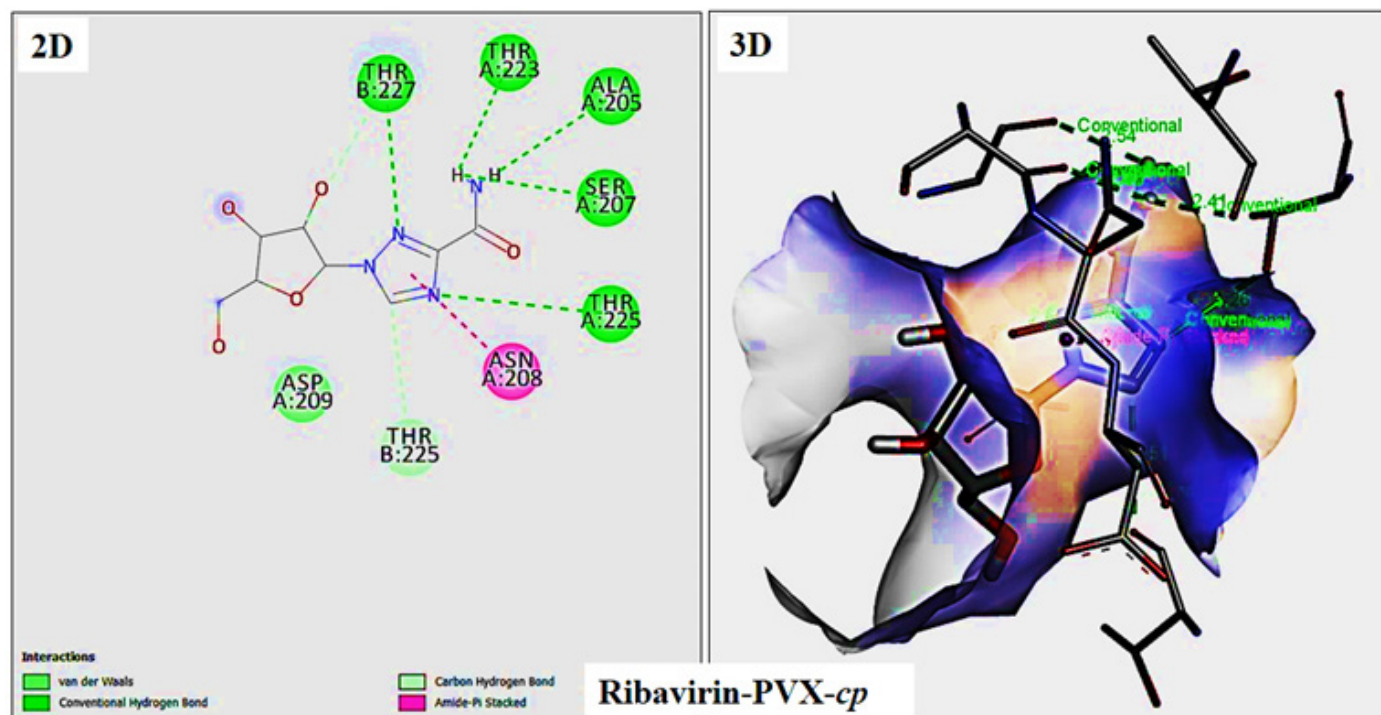


Figure 6: 2D and 3D structural analysis of Ribavirin (Control Ligand) binding to the PVX coat protein: insights into molecular interactions.

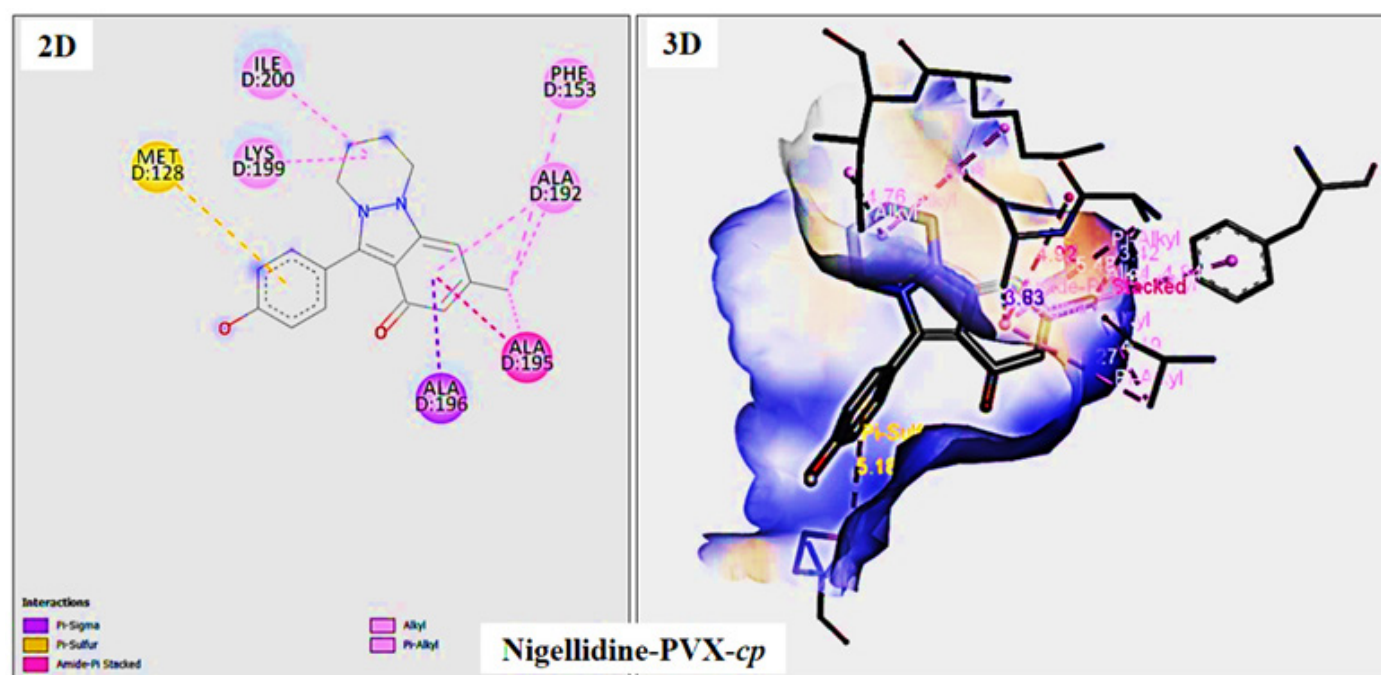


Figure 7: 2D and 3D structural analysis of Nigellidine ligand binding to the PVX coat protein: insights into molecular interactions.

Regarding common binding sites, ALA D:192 and ALA D:195 are frequently involved across multiple compounds, suggesting these sites are crucial for binding. LYS D:199 also appears often, highlighting its importance in the binding process. Black seed compounds, particularly Nigellidine and Nigellidine, show more hydrophobic and diverse interactions compared to Ribavirin, suggesting stronger binding

to non-polar regions of the PVX coat protein. The variety of bonds (e.g., Pi-Sigma, Pi-Alkyl, and Pi-Sulfur) across black seed compounds suggests complex and stable binding profiles, potentially offering more effective inhibition of the PVX coat protein.

In a conclusion, black seed compounds, especially Nigellidine and Nigellidine, form stronger and more

diverse interactions with the PVX coat protein than Ribavirin. These findings support the potential of black

seed compounds as effective antiviral agents, providing insights into their ability to inhibit viral replication.

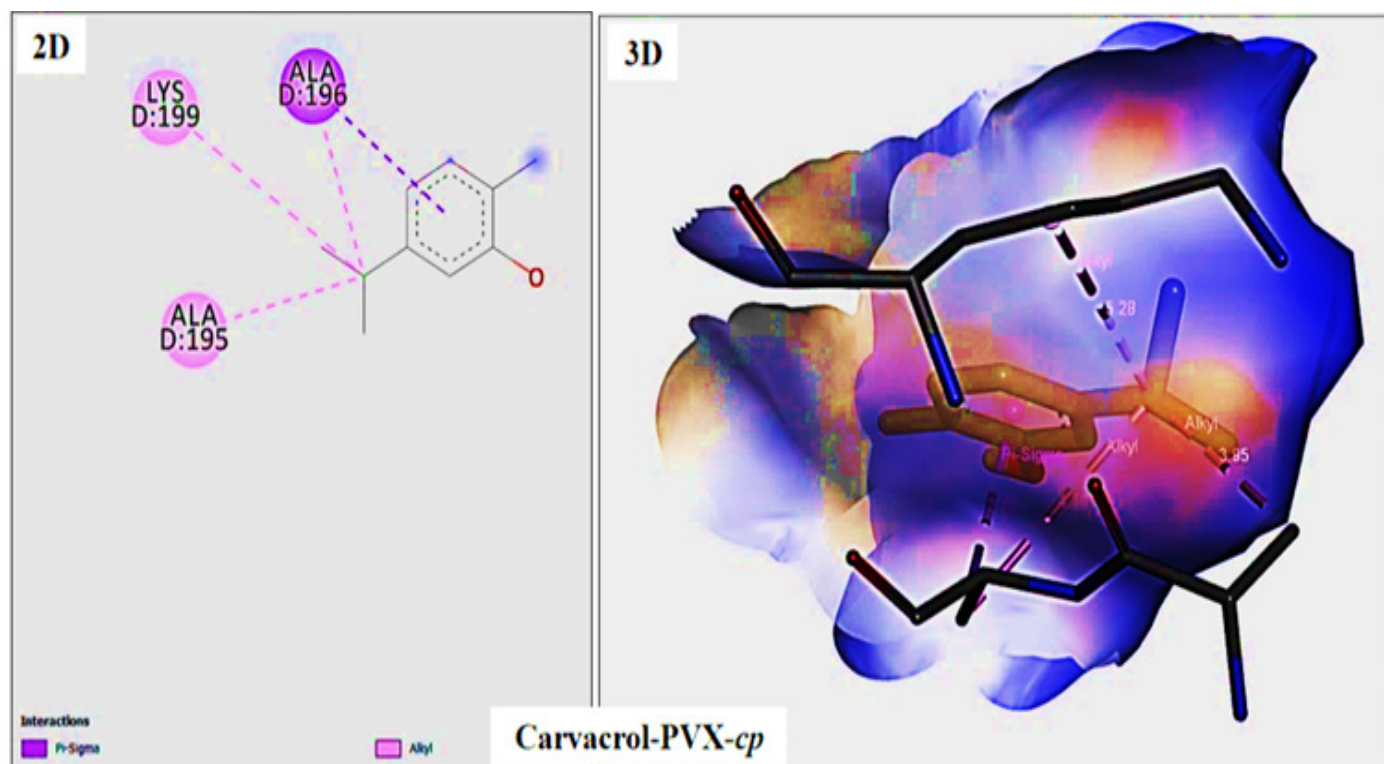


Figure 8: 2D and 3D structural analysis of Carvacrol ligand binding to the PVX coat protein: insights into molecular interactions.

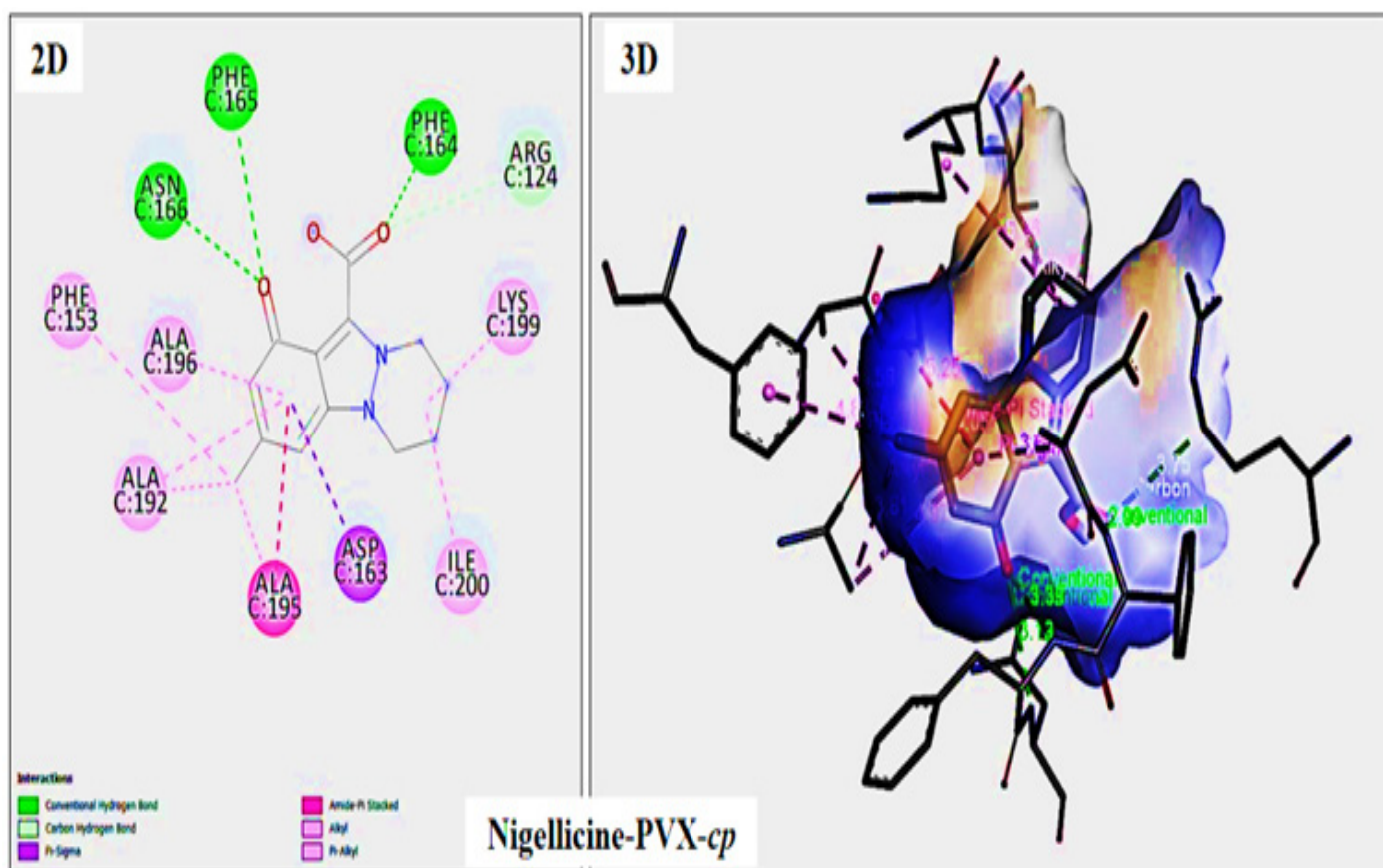


Figure 9: 2D and 3D structural analysis of Nigellidine ligand binding to the PVX coat protein: insights into molecular interactions.

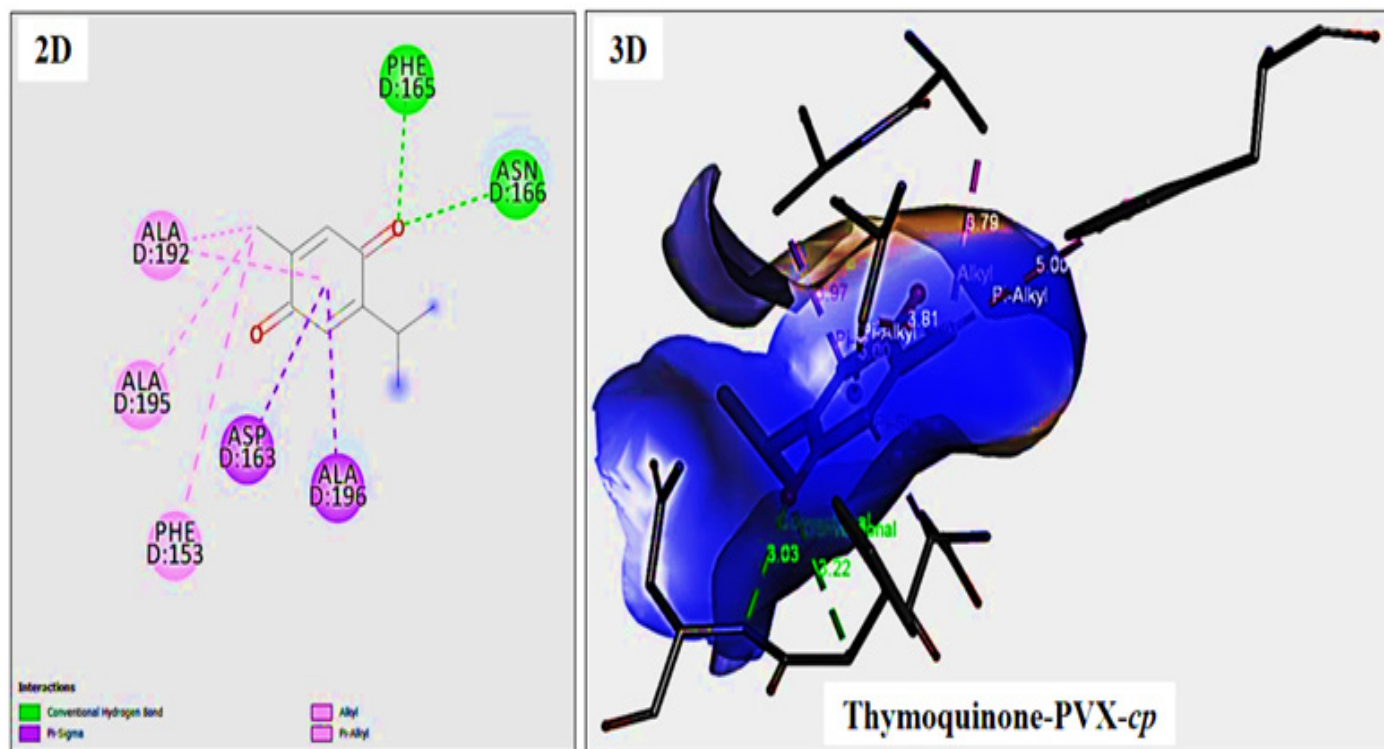


Figure 10: 2D and 3D structural analysis of Thymoquinone ligand binding to the PVX coat protein: insights into molecular interactions.

Discussion

It is important to note that drug design and molecular docking is a statistical analysis performed in the program design to study the binding ability between the compound and the protein, which is clarified through the results of binding affinity. The best compound is selected based on the RMSD result, which should be zero to achieve the best outcome. When building programs and servers, they are designed using precise mathematical and statistical equations to ensure their effectiveness and reliability. In this study a comparative analysis of the physicochemical and pharmacokinetic properties of the four selected compounds of black seed was conducted compared to Ribavirin as a control in a trial to pay an attention to the potential therapeutic benefits and limitations of these compounds, particularly when considering their drug-likeness and ADMET properties as recommended by Ejeh *et al.* (2021). This could be due to that Thymoquinone is non-toxic at low to moderate doses but requires caution at very high doses. It has shown promising therapeutic effects, including antioxidant, anti-inflammatory, and anticancer properties. Nigellidine appears non-toxic at typical pharmacological doses, but further research is needed for a comprehensive safety evaluation. Carvacrol is safe at typical

therapeutic doses, though higher doses may cause liver and gastrointestinal toxicity. Nigellidine seems to have low toxicity and is generally safe at moderate doses. At the level of molecular properties all black seed components fall within the optimal molecular weight range (<500 g/mol) for drug-likeness, similar to Ribavirin (Arrué *et al.*, 2022). Additionally, the low number of rotatable bonds in these components (1–3) suggests reduced molecular flexibility, which can enhance their ability to interact with biological targets. The reduced number of hydrogen bond donors and acceptors, compared to Ribavirin, might indicate improved membrane permeability, supporting their bioavailability. These results align with that reported by Lipinski *et al.* (1997) and Daina *et al.* (2017).

One of the most notable findings is the high GI absorption predicted for all black seed components. In contrast, Ribavirin exhibits low GI absorption, limiting its bioavailability when administered orally. High GI absorption makes black seed components, such as Thymoquinone, promising candidates for oral therapeutic formulations. Similar observations were reported by Daina *et al.* (2017), Mir *et al.* (2022) and Shoaib *et al.* (2023).

Regarding the blood-brain barrier (BBB), all black seed components are predicted to cross the BBB,

highlighting their potential for central nervous system (CNS) activity. Thymoquinone, in particular, has been extensively studied for its neuroprotective and anti-inflammatory effects, making this prediction clinically significant ([Woo et al., 2012](#); [Rahman et al., 2024](#)). Ribavirin, however, is not BBB permeable, limiting its use for CNS-related conditions.

The black seed components exhibit selective inhibition of specific cytochrome P450 enzymes. For instance, Carvacrol and Nigellidine inhibit CYP1A2, and Nigellidine inhibits CYP2D6. These interactions may result in potential drug-drug interactions when co-administered with other drugs metabolized by these enzymes. However, the absence of inhibitory activity for key enzymes such as CYP2C19, CYP2C9, and CYP3A4 across all compounds minimizes the risk of broad metabolic interference ([Guengerich, 2008](#)). In contrast, Ribavirin does not inhibit any cytochrome P450 enzymes, suggesting fewer concerns in this area.

All tested compounds in this study comply with Lipinski's Rule of Five, confirming their drug-likeness and potential for oral bioavailability ([Lipinski et al., 1997](#)). This suggests that black seed components possess favorable pharmacokinetic properties, making them viable candidates for further development.

Results of virtual screening and docking scores of ligands against PVX-replicase align with previous studies of [Imran et al. \(2022\)](#) that highlighted Nigellidine's efficacy as a lead compound for antiviral drug development. Further in vitro and in vivo studies are warranted to confirm the antiviral properties of Nigellidine and Nigelline against PVX and assess their potential as therapeutic agents.

The findings of docking scores and virtual screening of ligands (Carvacrol, Nigelline, Nigellidine, and Thymoquinone) against PVX-coat protein are in agreement with earlier research that emphasizes Nigellidine's robust molecular interactions and antiviral potential ([Abbas et al., 2024](#)). Future studies, including molecular dynamics simulations and experimental validation, are necessary to confirm these findings and establish the therapeutic efficacy of Nigellidine and Nigelline against PVX.

The experimental results provide valuable insights into the interaction profiles of black seed compounds-Carcacrol, Nigelline, Nigellidine, and

Thymoquinone-with the PVX replicase and coat proteins. These findings demonstrate the potential of these natural compounds as antiviral agents, suggesting they may inhibit viral replication through diverse and stable interactions with the viral proteins.

The interaction of black seed compounds with the PVX replicase protein reveals a complex binding mechanism. The ligands, especially Nigelline and Nigellidine, form multiple and diverse bonds, with interactions ranging from hydrophobic (Alkyl, Pi-Alkyl) to electrostatic (Pi-Cation, Pi-Donor Hydrogen, Conventional Hydrogen). These bonding profiles reflect the multifaceted interaction mechanisms that could contribute to the stability and affinity of the ligand-protein complex.

Ribavirin, as a control, forms three bonds with PVX replicase (Pi-Alkyl with LYS A:805, Pi-Cation with LYS A:741, and Pi-Donor Hydrogen with GLN A:832). This suggests that Ribavirin, a known antiviral agent, binds through relatively simple interactions that are likely primarily hydrophobic or electrostatic. These types of interactions have been previously documented as contributing to the inhibition of viral RNA replication in several viral systems, including those of RNA viruses like the Influenza virus and Hepatitis C virus ([Graci and Cameron, 2006](#); [Thomas et al., 2012](#)).

Carvacrol forms interactions with LYS (A:805), ASP (A:802), GLN (A:832), and LYS (A:741), involving Alkyl, Pi-Cation, Pi-Donor Hydrogen, and Pi-Alkyl bonds. These bond types indicate that Carvacrol engages the PVX replicase through both hydrophobic and electrostatic forces, similar to findings in other studies where Carvacrol exhibited antiviral properties by interacting with key viral enzymes (e.g., in HCV and HSV) ([Pilau et al., 2011](#)).

Nigelline and Nigellidine, both with complex interaction profiles (7 and 8 bonds, respectively), form a variety of interactions, including conventional hydrogen bonds (e.g., ARG A:867, GLY A:740, LYS A:741) and Pi-Alkyl interactions (e.g., TRP A:771, PHE A:771). These interactions suggest that these compounds may have a more stable and extensive binding profile compared to Ribavirin, likely contributing to stronger inhibition of PVX replicase. The inclusion of serine, threonine, and glycine residues in the binding interactions suggests

that these compounds may stabilize viral protein structures, which could be essential in inhibiting replication (Maideen, 2020; Basurra *et al.*, 2021).

Thymoquinone shares some similarities with Ribavirin in terms of the bond types (Alkyl, Pi-Cation, Pi-Donor Hydrogen) formed with LYS (A:805), LYS (A:741), and GLN (A:832). While these interactions are less complex than those seen with Nigellidine and Nigellidine, the distinct binding pattern of Thymoquinone suggests that it may also exert inhibitory effects through interactions that stabilize the PVX replicase structure. Previous studies have shown that Thymoquinone, derived from *Nigella sativa*, has antiviral activity against various viruses, likely through similar mechanisms of action (Shoaib *et al.*, 2023). Based on our docking studies, Nigellidine may exert its antiviral effects by binding to key viral proteins involved in viral replication, such as the PVX coat protein or RNA-dependent RNA polymerase. The interaction likely prevents the virus from binding to host cells or inhibits viral replication. Additionally, Nigellidine might exert an effect on the host cell machinery by modulating immune responses, such as enhancing interferon production or inhibiting viral proteases. Similarly, Nigellidine could interfere with viral entry or replication. Our analysis suggests that it may interact with viral RNA or disrupt the formation of viral complexes necessary for replication. Moreover, Nigellidine may enhance host antiviral responses, such as activating pathways involved in apoptosis or autophagy, which can limit viral spread.

Regarding the interactions with PVX coat protein the profiles of black seed compounds with the PVX coat protein are similarly diverse, with various bond types indicating that these compounds may disrupt viral particle assembly or stability, which is crucial for viral infectivity.

Ribavirin forms 8 bonds with the PVX coat protein, predominantly hydrophilic interactions (Conventional Hydrogen, Amide-Pi Stacked, Van der Waals), indicating a mechanism that likely targets hydrophilic regions of the protein. This is consistent with studies showing that Ribavirin can inhibit viral replication by interfering with viral protein synthesis and RNA replication (Parker, 2005; Paeshuyse *et al.*, 2011).

Carvacrol, with 3 hydrophobic bonds (Alkyl, Pi-

Sigma), interacts with non-polar regions of the PVX coat protein. This suggests that Carvacrol might interfere with viral coat assembly by targeting regions critical for protein-protein interactions during virion formation. Hydrophobic interactions are commonly involved in disrupting viral protein conformations and preventing viral particle assembly (Sánchez *et al.*, 2015; Sharifi-Rad *et al.*, 2018).

Nigellidine forms 11 bonds with a range of hydrophobic (Alkyl, Pi-Sigma, Pi-Alkyl) and electrostatic (Conventional Hydrogen, Amide-Pi Stacked) interactions, suggesting that Nigellidine could bind to multiple regions of the coat protein, affecting its conformation and potentially disrupting its ability to form a stable viral shell. The wide variety of bonds formed by Nigellidine enhances its potential as an antiviral agent (Islam *et al.*, 2021).

Nigellidine also shows diverse interactions, including Pi-Sulfur with MET D:128, which could further enhance its binding affinity. Pi-Sulfur interactions are important in stabilizing interactions with sulfur-containing residues, making this bond type crucial in drug design for increasing the binding strength (Mustafa and Winum, 2022).

Thymoquinone's interaction with the PVX coat protein is dominated by a mixture of hydrophobic (Pi-Alkyl, Alkyl) and hydrophilic (Conventional Hydrogen, Pi-Sigma) bonds. This combination suggests that Thymoquinone may bind to both hydrophobic and hydrophilic regions of the protein, potentially leading to the destabilization of the viral structure (Tania *et al.*, 2021).

Regarding the common binding sites and potential for inhibition, amino acids, such as ALA D:192, ALA D:195, and LYS D:199, appear frequently across several ligands, suggesting that these regions play a significant role in the binding of black seed compounds to both the PVX replicase and coat proteins. The common involvement of these sites across multiple compounds supports their importance in the viral protein's structural stability and function.

The hydrophobic interactions observed in black seed compounds, particularly Nigellidine and Nigellidine, suggest that these compounds may preferentially bind to non-polar regions of the viral proteins, interfering with their assembly or conformational stability.

Such interactions have been shown to be crucial for disrupting the structural integrity of viral proteins (Badani *et al.*, 2014).

To clarify the novelty of the study, the following aspects are highlighted: **Unique Set of Compounds:** Unlike previous studies, our work focuses on a broader range of bioactive compounds derived from *Nigella sativa*, including some that have not been extensively studied in the context of antiviral activity against PVX. **Antiviral Focus:** While many previous studies on *Nigella sativa* compounds have primarily investigated antibacterial or anticancer properties, our study specifically explores the antiviral potential against PVX, which, to our knowledge, has not been extensively explored in similar molecular docking studies. **Methodology Enhancements:** We employed advanced molecular docking techniques, utilizing newer algorithms or updated databases that may not have been included in earlier studies. Additionally, we used a more comprehensive set of criteria to evaluate the binding affinity and stability of compounds to PVX targets.

There are currently few antiviral treatments for PVX in plants, with most strategies focusing on prevention, such as using resistant varieties, cultural practices, and controlling aphid vectors. Chemical treatments are limited, and research into antiviral agents for plant viruses is still emerging. In contrast, Nigellidine and Nigellicine offer a novel approach, with *in silico* findings suggesting they could inhibit viral replication or disrupt viral-host interactions. These natural compounds provide a more eco-friendly, sustainable alternative to chemical treatments, with promising results from other studies. If experimentally validated, they could complement or even replace existing PVX management strategies, offering a new antiviral treatment option.

Experimental validation: We also plan to experimentally validate the results predicted by the *in silico* approach, which will provide an essential layer of confidence in our findings. This experimental validation is often lacking in many studies that focus solely on molecular docking. By integrating both computational predictions and experimental data, we aim to strengthen the reliability and robustness of our conclusions, ensuring that the proposed compounds, Nigellidine and Nigellicine, are not only theoretically effective but also practically viable in real-world

applications. This combined approach will help bridge the gap between computational modeling and actual biological outcomes, offering a more comprehensive understanding of the compounds' potential.

Limitations of molecular docking approaches

Molecular docking approaches, while powerful, have several limitations that can affect the accuracy and interpretation of results. The reliability of docking predictions is highly dependent on the scoring function used, which may not fully capture all aspects of molecular interactions, such as solvation effects or entropic contributions. Additionally, many docking methods assume rigid receptor and ligand structures, neglecting the conformational flexibility of both, which can impact prediction accuracy. Furthermore, the quality of protein-ligand interaction models, including hydrogen bonding and electrostatic interactions, may not always be accurately represented. Missing water molecules, ions, and oversimplified solvation models can also affect results. Moreover, docking typically involves limited sampling of ligand conformations and binding modes, potentially missing key interactions. The lack of consideration for the dynamic biological context, including protein-protein interactions and cellular conditions, further limits the scope of docking predictions. These factors must be taken into account when interpreting docking results, and experimental validation remains crucial for confirming their relevance.

In our point of view, the findings of this study not only enhance our understanding of the molecular mechanisms behind viral infections in plants but also open up new possibilities for developing innovative, environmentally friendly approaches to plant protection. These strategies could significantly reduce the impact of viral diseases on crops, thereby supporting food security and sustainable agriculture.

While the potential for developing antiviral compounds based on the findings of this study is high, overcoming these challenges will require further research, technological advancements, and close collaboration between researchers, industry stakeholders, and regulatory bodies. Addressing these issues will be essential for translating these compounds from the laboratory to practical, field-ready treatments that can contribute to sustainable plant protection.

To experimentally validate the antiviral compounds, we propose several approaches, including *in vitro* enzyme inhibition assays, plant cell-based assays (e.g., using tobacco protoplasts), and whole plant infection studies in tobacco (*Nicotiana tabacum*), and tomato (*Solanum lycopersicum*). These models are suitable for evaluating antiviral efficacy, viral load reduction, and potential phytotoxicity. Additionally, greenhouse and field trials will be necessary to assess the real-world effectiveness and safety of the compounds. This multi-step validation process will help confirm the practical applicability of the proposed treatments for plant viral diseases.

To validate the computational predictions of antiviral compounds, several key assays can be used. Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC) can confirm binding affinity and thermodynamics between the compound and viral protein. Enzyme inhibition assays, such as RNA-dependent RNA polymerase (RdRp) inhibition, will test the functional impact of the compounds. Quantitative PCR (qPCR) and Western Blot can measure viral load and protein levels in treated plants, confirming the reduction in viral replication. Additionally, Luciferase Reporter Assays and plant symptom scoring will help assess antiviral efficacy in plant models, while toxicity assays will ensure the compounds are safe for plants. These assays provide comprehensive validation of computational predictions and the compounds' effectiveness.

Conclusions and Recommendations

This study underscores the potential of *Nigella sativa* bioactive compounds as natural antivirals against PVX. *In silico* predictions, supported by experimental validation, reveal that compounds like Nigellidine and Nigellidine demonstrate strong binding affinity to both PVX replicase and coat proteins. Their diverse interaction profiles, involving various bond types, suggest they could effectively inhibit viral replication and assembly. Additionally, the favorable pharmacokinetic and physicochemical properties of black seed compounds, including improved oral bioavailability and potential CNS activity, make them promising therapeutic agents. These findings advocate for further exploration of black seed compounds in preclinical and clinical studies, offering a plant-based solution to combat viral infections in agriculture. The revised conclusion emphasizes that while our

study provides valuable insights into the antiviral potential of these compounds, further *in vitro* and *in vivo* research is required to confirm their efficacy and safety. It is also clarified that, while molecular docking and ADMET analyses show promise, the compounds' actual antiviral activity in plants needs validation through experimental methods like viral replication assays and plant infection models.

To validate our *in silico* findings, the next step is to conduct *in vitro* antiviral assays using PVX-infected plant models. Plants will be treated with varying concentrations of Nigellidine and Nigellidine, and viral load will be measured using qRT-PCR or ELISA to evaluate the inhibition of viral replication. These assays will provide direct evidence of the compounds' effectiveness in preventing or reducing PVX infection. Additionally, we recommend the need to assess the cytotoxicity of Nigellidine and Nigellidine in plants. Moreover, we recommend performing several cytotoxicity studies, including MTT assays, chlorophyll content measurements, and plant growth evaluations, to determine any potential toxicity and establish the therapeutic window for these compounds.

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Novelty Statement

This study is the first to explore *Nigella sativa* bioactive compounds against Potato virus X using molecular docking and providing novel insights into plant-derived antiviral agents for sustainable virus management.

Author's Contribution

Adham Ezz El-Regal Mahmoud: Methodology, investigations, data analysis and writing the original draft

Atef Shoukry Sadik: Supervision, research design, data analysis, validation of results and writing the original draft

Ahmed Mahdy: Methodology, investigation,

validation of results and writing review & editing

Ethical approval

Ethical approval is not required for this study as it is purely computational and does not involve human subjects, animals, or live plants.

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Conflict of interests

The authors have declared no conflict of interests.

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