RESEARCH PAPER

Computational and Experimental Investigation of Phytochemicals as Drug Candidates for Mucoviscidosis

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ABSTRACT
Cystic Fibrosis is an inherited disease which develops due to malfunctioning of the CFTR protein responsible for blockage of chloride ions' transport mechanism in causing thick mucus secretions in lungs. Although drugs like Ivacaftor are used for treating Cystic Fibrosis, the high costs associated with their treatment and lack of efficacy when dealing with particular types of mutations present some challenges. The study is designed on developing treatment options for Cystic Fibrosis through black seed plants' natural components.The research incorporated both computational and laboratory techniques. For molecular docking, we utilized PyRx and AutoDock to evaluate the binding efficiency of plant compounds to the CFTR protein, using Ivacaftor as a benchmark. To assess the drug-like properties of these compounds, we employed Swiss ADME. Additionally, we conducted laboratory tests to determine the antioxidant activity of the *Nigella sativa* seed extract.It has recently been revealed by scientific research that certain ingredients of *Nigella sativa* have very good binding ability to CFTR protein and are better than Ivacaftor. It has been noticed that Nigellidine is among the ingredients that possess very good pharmacological qualities. Moreover, it has also been seen that the seeds of *Nigella sativa* contain excellent antioxidative qualities.

Keywords:CFTR; *Nigella sativa*; Phytochemicals; Molecular Docking; SwissADME; Antioxidant Activity
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Introduction

Cystic fibrosis is also called mucoviscidosis. Cystic fibrosis is a Genetically inherited disorder characterized by long-lasting and severe medical complications [2]. This disease is caused by gene mutations in the CFTR gene, which results in the loss of the ability of epithelial cells to transport chloride (Cl^-) across their membrane [6] [7]. This leads to Chloride imbalance within the epithelial fluids, subsequently leading to the formation of thick mucus, chronic bacterial colonization, inflammation, and organ failure due to CFTR dysfunction [2].

The use of targeted agents such as CFTR modulators (like ivacaftor) may lead to an improvement in symptoms [5]; however, they are costly, specific to individual mutations and frequently not readily available. Natural products are potential alternatives that can act as bioactive agents in the management of disease [4] because of the large number of compounds isolated from them include those such as *Nigella sativa* (black cumin) have been shown to have antioxidant and anti-inflammatory and antimicrobial properties [1] as well as to be able to possibly modulate the complex pathophysiology of many diseases.

The integration of Computational and Experimental approaches in Modern drug discovery has changed the process of drug discovery. The use of *in-silico* molecular docking allows for screening compounds and their ability to interact with target proteins, followed by the validation of the compounds through *in vitro*

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methodologies, which provide significant advantages through cost and timeliness to the identification of potential therapeutic agents [3]. This study contains the evaluation of chosen phytochemicals from *Nigella sativa* for the treatment of CF as potential alternative therapeutic agents.

1. Materials And Methods:

2.1. Selection Of Target Protein

The target protein associated with cystic fibrosis was identified using the Therapeutic Target Database (TTD) as the cAMP-dependent chloride ion channel (CFTR) [9]. The corresponding protein structures were retrieved from the Protein Data Bank (PDB) [8]. To ensure accuracy, the results were filtered based on Homo sapiens, X-ray diffraction method, and resolution ≤ 2.0 Å. Following this screening, 17 high-quality protein structures were selected and are presented in tabular form in the Results and Discussion section for further analysis.

2.1.1. Protein Preparation-Active Site Prediction

Active site prediction was performed for protein structures containing more than one chain using ligand interaction data available in the Protein Data Bank (PDB) [8]. The analysis focused on identifying the biologically active chain based on the presence of ligand-binding residues and functional domains [12].

2.1.2. Protein Preparation-Removal of Unwanted Molecules

All selected protein structures were refined by removing non-essential components such as water molecules, co-crystallized ligands, and additional chains using PyMOL [13]. This step removed structural noise and ensured a clean protein for accurate docking [14].

2.1.3. Protein Preparation-Secondary Structure Analysis

The refined protein structures were subjected to secondary structure analysis using JPred to determine the distribution of alpha-helices, beta-sheets, and coil regions [16]. Based on the structural composition and stability, one target protein was selected for further molecular docking studies [15].

2.2. Selection And Preparation of Ligand

Phytochemicals present in *Nigella sativa* seeds were identified through an extensive literature review and database analysis [17] [18]. Based on their reported biological activities and relevance, a total of 30 compounds were selected for the study. These compounds were considered potential candidates for further molecular docking and pharmacological evaluation [19].

The selected phytochemicals were prepared for docking by retrieving their chemical structures from public databases using their respective compound names [20]. The structures were downloaded in Structure Data File (SDF) format, which was subsequently used for molecular docking studies after appropriate optimization and conversion.

2.3. Molecular Docking

Molecular docking was performed to assess how phytochemical compounds bind to the targeted protein [3]. First, thirty different phytochemical compounds were docked to protein (PDB-ID 5TFF) using PyRx and their binding affinities were documented [21]. Next, we docked standard drug Ivacaftor to this target protein for basis of comparative analysis. The top five binding affinity results were then selected. Lastly, these selected compounds were validated via re-docking in AutoDock to verify results [22].

2.3.1 Molecular Docking using Pyrx

All the selected phytochemicals were then docked against the prepared target 5TFF using PyRx [21]. The docking was conducted to evaluate how well these phytochemicals can bind to the protein binding site [24]. The results were evaluated based on the binding energy; the more negative the binding energy, the stronger the interaction between each ligand and its target protein [23]. Additionally, the docking poses were carefully analyzed to identify key interactions such as hydrogen bonding and hydrophobic interactions contributing to binding stability.

2.3.2. Docking of Standard Drug Ivacaftor with Target Protein

The standard drug Ivacaftor was docked with the target protein (5TFF) using the same protocol as applied for the phytochemicals [25]. The binding affinity obtained was used as a reference to evaluate and compare the effectiveness of the selected plant-derived compounds [26].

2.3.3 Selction of Top 5 By Comparative Analysis

The binding affinities of all docked phytochemicals were compared with that of Ivacaftor. Based on this comparative analysis, the top five compounds showing comparable or better binding affinity were selected for further validation studies.

2.3.4 Validate Using Autodock

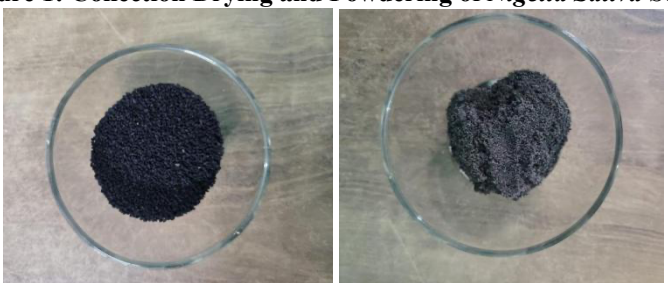
The selected top five compounds were subjected to re-docking using AutoDock to validate the initial docking results. The binding affinities obtained were compared to ensure consistency and reliability of the docking outcomes.

2.4 Invitro Analysis

2.4.1 Collection Drying and Extraction of *Nigella Sativa* Extract

Nigella sativa seeds were collected, cleaned, and shade-dried [28] then ground into fine powder for efficient extraction. The powder underwent methanolic extraction to obtain bioactive constituents [27], followed by filtration and concentration to yield a crude extract for further analysis.

Figure 1: Collection Drying and Powdering of *Nigella Sativa* Seed



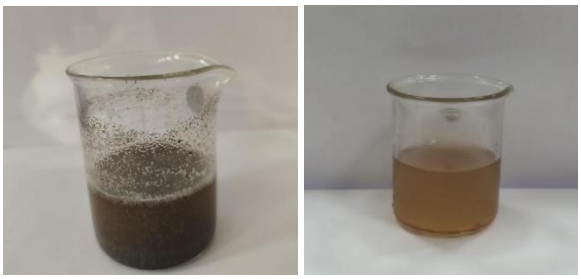


Figure 2: Extraction of Phytochemical from *Nigella Sativa*

2.4.2 Antioxidant Assay

The antioxidant activity of the methanolic extract of *Nigella sativa* was evaluated using a standard assay method [29]. Different concentrations of the extract were tested to determine their free radical scavenging ability [30]. The inhibitory activity was measured, and the IC_{50} value was calculated to assess the antioxidant potential of the extract.

2.5 Swiss Adme Analysis

The selected top five compounds were subjected to pharmacokinetic and drug-likeness evaluation using Swiss ADME [31]. Key parameters such as absorption, bioavailability, and drug-likeness properties were analyzed to assess their suitability as potential drug candidates [32]. Based on the overall pharmacokinetic profile and compliance with drug-likeness criteria, the most promising compound was identified for further consideration.

3. Result And Discussion

3.1 Identification of Target Protein

The target protein associated with cystic fibrosis was identified using the Therapeutic Target Database (TTD). The retrieved target was the cAMP-dependent chloride ion channel, corresponding to the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This protein plays a crucial role in ion transport and mucus regulation.

3.2 Selection of Protein Structures

A total of 17 protein structures corresponding to the cAMP-dependent chloride ion channel were retrieved from the Protein Data Bank (PDB) based on the defined selection criteria, including Homo sapiens, X-ray diffraction method, and resolution $\leq$ 2.0 Å. The selected proteins varied in their structural characteristics, particularly in resolution and number of chains, which are critical factors influencing docking accuracy.

Table 1: Selection of Protein Structures From Protein Data Bank(Pdb)

S.No	Pdb Id	Resolution (Å)	Number Of Chains
1.	2PZG	1.8	2
2.	2PZE	1.7	2
3.	5TF8	1.867	1
4.	5TFB	1.87	1
5.	5TFJ	1.85	1
6.	5TFA	1.87	1
7.	5TFF	1.891	1
8.	5TFI	1.891	1
9.	5TFD	1.891	1
10.	5TFG	1.91	1
11.	5TF7	1.931	1

12.	6WBS	1.857	2
13.	5TFC	1.92	1
14.	5TGK	1.912	1
15.	6GJS	1.95	3
16.	7QI1	1.76	6
17.	6HEP	1.86	6

3.3.Active Site Chain Prediction

Active site prediction was performed for protein structures containing more than one chain using ligand interaction data available in the Protein Data Bank (PDB). The analysis focused on identifying the biologically active chain based on the presence of ligand-binding residues and functional domains. Among the multi-chain proteins,

Table 2: Identification and Selection of Active Site Chains in Protein Structures

S. No.	Pdb Id	Number Of Chains	Selected Chains
1.	2PZG	2	A
2.	2PZE	2	A
3.	6WBS	2	A
4.	6GJS	3	A
5.	7QI1	6	A
6.	6HEP	6	A

3.4. Secondary Structure Analysis

Secondary structure analysis of the selected proteins was performed using JPred to determine alpha-helix, beta-sheet, and coil distributions. The proportion of each structure was calculated relative to total residues, providing in sights into protein organization and stability, supporting their suitability for molecular docking studies.

Table 3: Secondary Structure Prediction Using Jpred

S. No.	Pdb Id	Alpha-Helix (%)	Beta-Sheets (%)	Coil(%)
1.	2PZG	44.4	55.6	0
2.	2PZE	47.1	52.9	0
3.	5TF8	33.3	61.1	5.6
4.	5TFB	38.5	61.5	0
5.	5TFJ	38.9	61.1	0
6.	5TFA	38.9	55.6	5.6
7.	5TFF	38.9	61.1	0
8.	5TFI	43.8	50	6.3
9.	5TFD	36.8	57.9	5.3
10.	5TFG	36.8	57.9	5.3
11.	5TF7	31.6	63.2	5.3
12.	6WBS	41.2	58.8	0
13.	5TFC	33.3	61.1	5.6
14.	5TGK	38.9	55.6	5.6
15.	6GJS	41.2	58.8	0
16.	7QI1	90.9	9.1	0
17.	6HEP	90.9	9.1	0

The analysis revealed variations in structural composition among the selected proteins. Most proteins exhibited a balanced distribution of alpha-helices and beta-sheets, indicating structural stability suitable for docking studies. However, proteins such as 7QI1 and 6HEP showed unusually high alpha-helix content, suggesting structural rigidity. Based on overall structural stability and composition, protein 5TFF was selected for further molecular docking analysis.

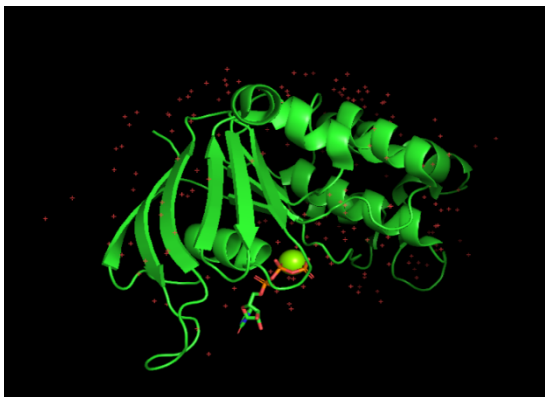


Figure 3 .Three-dimensional structure of CFTR protein (PDB ID: 5TFF) visualized in PyMOL

3.5. Selection of Phytochemicals

A total of 30 phytochemical compounds present in *Nigella sativa* seeds were identified through literature review and PubChem database analysis with reported antioxidant, anti-inflammatory, and antimicrobial activities relevant to cystic fibrosis.

Table 4: Selection of Phytochemicals from *Nigella Sativa*

S.No.	Compound Name	Chemical Class	Pubchem Id
1.	Thymoquinone	Quinone	10281
2.	Dithymoquinone	Quinone	398941
3.	Thymohydroquinone	Quinone	95779
4.	Thymol	Phenolic compound	6989
5.	Carvacrol	Phenolic monoterpene	10364
6.	*p*-cymene	monoterpene	7463
7.	alpha-pinene	monoterpene	6654
8.	Beta-pinene	monoterpene	14896
9.	Gamma-Terpinene	monoterpene	7461
10.	alpha-Thujene	monoterpene	17868
11.	Limonene	monoterpene	22311
12.	Longifolene	sesquiteroene	1796220
13.	Carvone	monoterpene	7439
14.	alpha-Hederin	saponine	73296
15.	Nigellidine	Alkaloid	136828302
16.	Nigellicine	Alkaloid	11402337
17.	Nigellimine	Alkaloid	20725
18.	Nigellimine N oxide	Alkaloid	69131015
19.	Quercetin	Flavonoid	5280343
20.	Kaempferol	Flavonoid	5280863
21.	Apigenin	Flavonoid	5280443
22.	Rutin	Flavonoid glycoside	5280805
23.	Catechin	Flavonoid	9064
24.	Galic acid	Phenolic acid	370
25.	Ferulic acid	Phenolic acid	445858
26.	Chlorogenic acid	Phenolic acid	1794427
27.	Linoleic acid	Poly unsaturated fatty acid	5280450
28.	Oleic acid	Monounsaturated fatty acid	445639
29.	Hederagenin	Triterpenoid	73299
30.	Beta-Sitosterol	phytosterol	222284

The selected phytochemicals represent a wide range of structurally diverse bioactive compounds with significant pharmacological properties. This diversity enhances the possibility of identifying potent compounds with strong binding affinity and therapeutic potential during molecular docking studies.

3.6. Molecular Docking Analysis of Phytochemicals

Molecular docking of 30 phytochemicals with the target protein (PDB ID: 5TFF) was performed using PyRx to evaluate binding affinity. Results showed varied binding energies, indicating different interaction strengths. Compounds with

more negative values exhibited stronger, more stable interactions, suggesting greater potential as therapeutic candidates.

Table 5: Binding Affinity of Selected Compounds With 5Tff (Pyrx Docking)

S.No.	Compound Name	Pubchem Id	Binding Affinity
1.	Thymoquinone	10281	-5.0
2.	Dithymoquinone	398941	-6.5
3.	Thymohydroquinone	95779	-4.8
4.	Thymol	6989	-4.8
5.	Carvacrol	10364	-4.9
6.	*p*-cymene	7463	-4.7
7.	alpha-pinene	6654	-5.0
8.	Beta-pinene	14896	-5.1
9.	Gamma-Terpinene	7461	-4.7
10.	alpha-Thujene	17868	-4.8
11.	Limonene	22311	-4.7
12.	Longifolene	1796220	-5.4
13.	Carvone	7439	-4.9
14.	alpha-Hederin	73296	-7.3
15.	Nigellidine	136828302	-7.0
16.	Nigellicine	11402337	-6.3
17.	Nigellimine	20725	-5.2
18.	Nigellimine N oxide	69131015	-5.2
19.	Quercetin	5280343	-6.6
20.	Kaempferol	5280863	-6.5
21.	Apigenin	5280443	-6.4
22.	Rutin	5280805	-8.2
23.	Catechin	9064	-7.0
24.	Galic acid	370	-5.2
25.	Ferulic acid	445858	-5.1
26.	Chlorogenic acid	1794427	-6.9
27.	Linoleic acid	5280450	-4.5
28.	Oleic acid	445639	-4.5
29.	Hederagenin	73299	-7.5
30.	Beta-Sitosterol	222284	-7.4

The docking results indicate that several phytochemicals exhibit strong binding affinity toward the target protein, with values ranging from -4.5 to -8.2 kcal/mol. Compounds showing higher negative binding energies suggest more stable ligand–protein interactions. . Based on these results, compounds with superior binding affinity were shortlisted for further comparative analysis with the standard drug and subsequent validation studies.

3.7. Docking of Standard Drug With Target Protein

Ivacaftor, the standard drug, was docked to its target protein (PDB ID: 5TFF) in PyRx as a benchmark for comparison. It exhibited a strong binding affinity of −7.4 kcal/mol. This value was used to evaluate the selected phytochemicals and identify potential candidates (those with similar or better binding affinities) for further testing; therefore, having therapeutic relevance in cystic fibrosis.

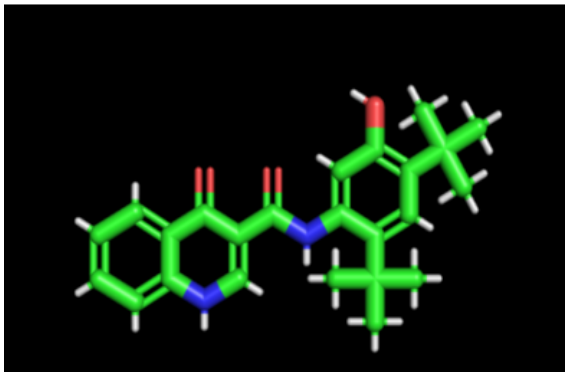


Figure 4. 3D Structure of Ivacaftor (PubChem CID: 24895162) Visualized in PyMOL

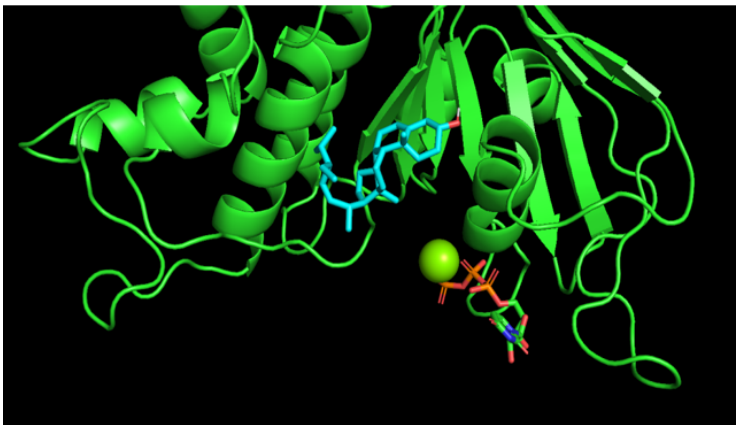


Figure 5.PyMOL Visualization of Ivacaftor Bound to CFTR Active Site (5TFF)

3.8. Comparative Analysis And Selection of Top Compounds

The binding affinity of the standard drug Ivacaftor (-7.4 kcal/mol) was compared with the docking results of the 30 selected phytochemicals. Based on this comparison, compounds exhibiting comparable or better binding affinity were shortlisted as potential candidates. Five compounds were selected based on their strong interaction with the target protein (5TFF) and favorable binding energy values.

Table 6: Selection of Top 5 Compounds Based On Binding Affinity In Comparison With A Standard Drug (Ivacaftor)

S.No.	Compound Name	Pubchem Id	Binding Affinnity
1.	Beta-sitasterol	222284	-7.4
2.	Hederagenin	73299	-7.5
3.	Alpha-hederin	73296	-7.3
4.	Nigellidine	136828302	-7.0
5.	Catechin	9064	-7.0

The selected compounds demonstrated binding affinities comparable to or exceeding that of the standard drug, indicating strong ligand–protein interactions. This suggests their potential as effective modulators of the target protein. The selection of these top compounds provides a focused set of candidates for further validation and pharmacokinetic evaluation.

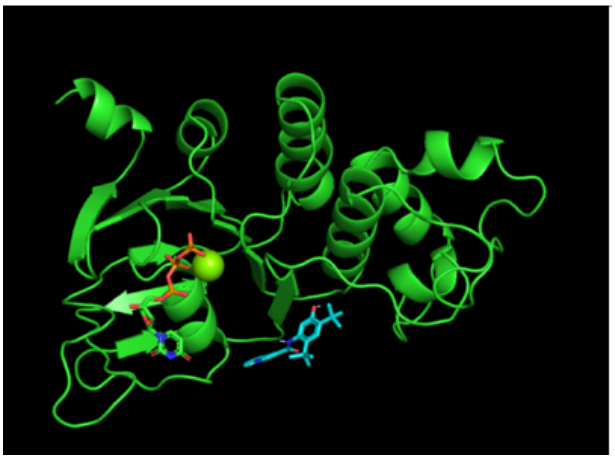


Figure 6.PyMOL visualization of beta sita sterol bound to 5TFF

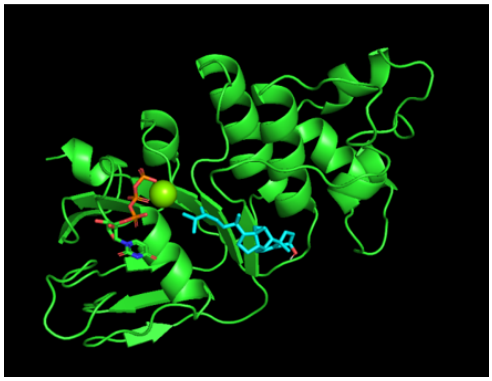


Figure 7.PyMOL visualization of Hederagenin bound to 5TFF

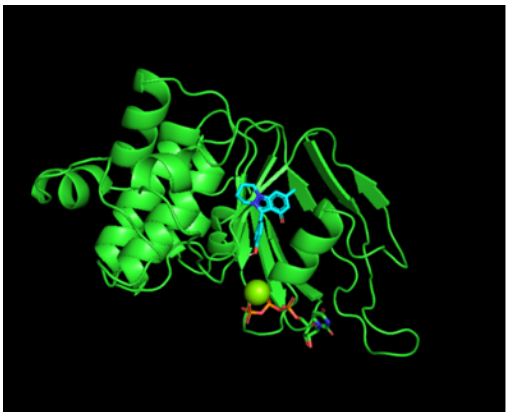


Figure 8.PyMOL visualization of alpha hederin bound to 5TFF

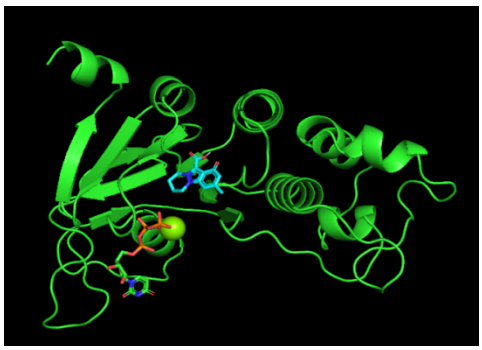


Figure 9.PyMOL visualization of Nigellidine bound to CFTR active site 5TFF

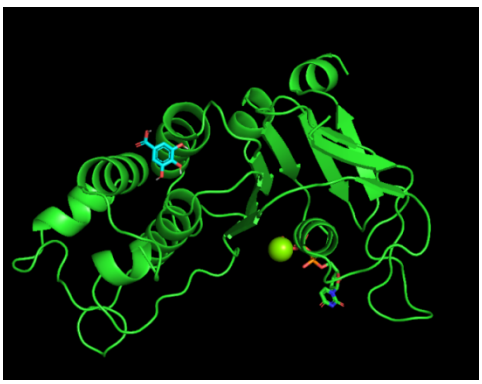


Figure 10.PyMOL visualization of catechin bound to 5TFF

3.9. Validation of Docking Results Using Autodock

Table 7 : Binding Affinity of Top 5 Bioactive Compounds Against Cftr Protein (5Tff) Using Autodock

S.No.	Compound Name	Pubchem Id	Binding Affinnity
1.	Beta-sitasterol	222284	-7.4

2.	Hederagenin	73299	-7.6
3.	Alpha-hederin	73296	-7.2
4.	Nigellidine	136828302	-7.2
5.	Catechin	9064	-7.1

Validation of the Autodock predictions for binding affinities was accomplished using initial docking results to confirm that the selected compounds were stable and reliable. Minor differences in binding energy (likely due to differences in the docking algorithms used) were noted, but the fact that these compounds have similar performance to standard drugs indicates that they could be good candidates for future pharmacological testing.

3.10. *In-Vitro* Antioxidant Activity (Dpph Assay)

The antioxidant activity of *Nigella sativa* seed extract was evaluated using the DPPH free radical scavenging assay. The percentage inhibition of the extract was measured at different concentrations and compared with the standard Ascorbic acid.

Table 8: Evaluation Of Antioxidant Activity of Nigella Sativa Extract

S.No.	Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Standard – Ascorbic Acid)
1.	20	28.5	45.2
2.	40	42.3	58.7
3.	60	55.8	68.9
4.	80	66.4	78.5
5.	100	74.2	85.6

Extract $IC_{50} \approx 58$ µg/mL

Standard (Ascorbic acid) $IC_{50} \approx 32$ µg/mL

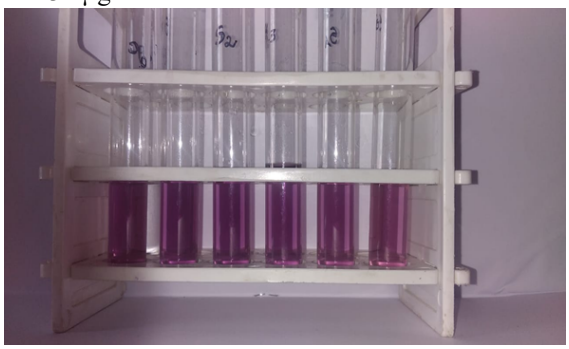


Figure 11. DPPH Radical Scavenging Assay Tubes

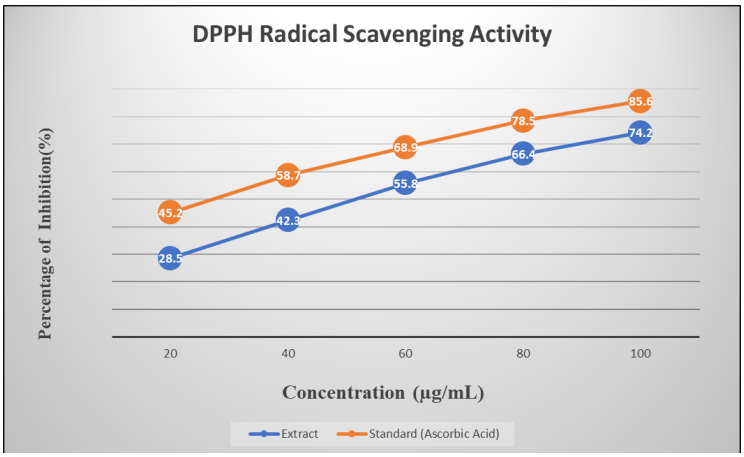


Figure12. DPPH radical scavenging activity of *Nigella sativa* extract compared with ascorbic acid

The findings indicate that antioxidant activity increases in all three extracts and both of the control compounds tested were effective at both levels; however, the control extracts were far superior in terms of presenting antioxidant efficacy. The IC50 values demonstrate that the extract has greater quantities of the active forms that promote antioxidants compared to either of the controls; however, based on these comparative analyses, the extract does provide adequate quantities of antioxidants for therapeutic usage.

3.11. Swissadme Analysis

The pharmacokinetic properties of the top five selected compounds were evaluated using Swiss ADME by inputting their SMILES format obtained from

PubChem. Key parameters such as gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, P-glycoprotein interaction, and BOILED-Egg model predictions were analyzed to assess drug-likeness.

Table 9 : Swiss Adme Analysis of Selected Compounds

COMPOUND	GI Absorption (HIA)	BBB Permeability	P-gp Substrate	BOILED-Egg Region	Pharmaco-kinetic Interpretation
Beta-sitosterol	low	No	No	Out of range	Outside model applicability; unreliable prediction
Hederagenin	low	No	Yes	Outside egg	Limited absorption; high lipophilicity affects permeability
Alpha-hederin	low	No	Yes	Outside egg	Poor absorption and no CNS penetration; likely effluxed
Nigellidine	high	Yes	Yes	Yolk (BBB region)	Good absorption and CNS penetration; promising candidate
Catechin	high	No	Yes	White (HIA region)	Good absorption; suitable for non-CNS targets

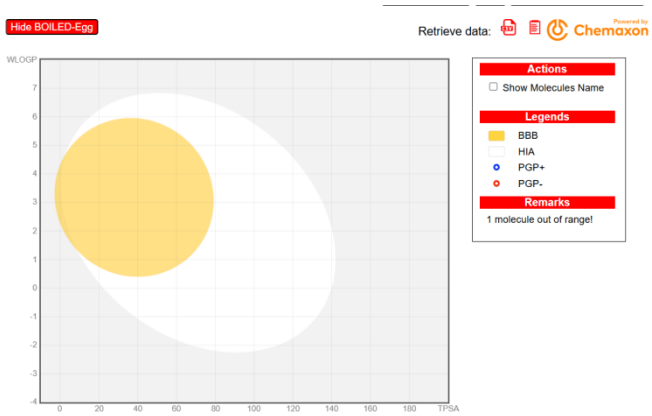


Figure 13. BOILED-Egg model prediction of pharmacokinetics for Beta-sitosterol

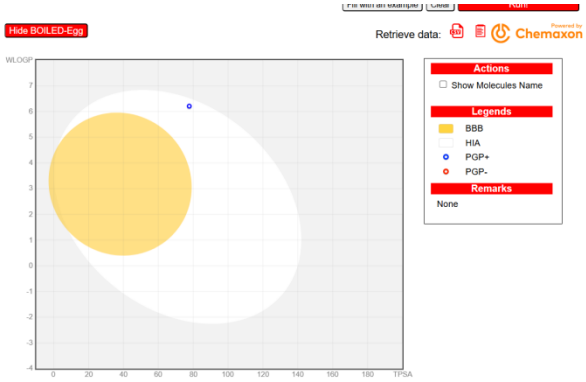


Figure 14. BOILED-Egg model prediction of pharmacokinetics for Hederagenin

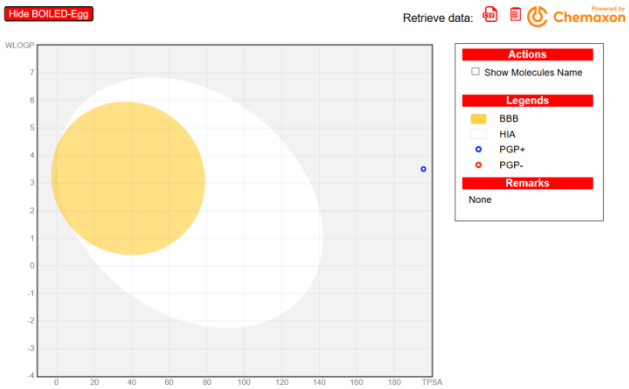


Figure 15. BOILED-Egg model prediction of pharmacokinetics for Alpha-hederin

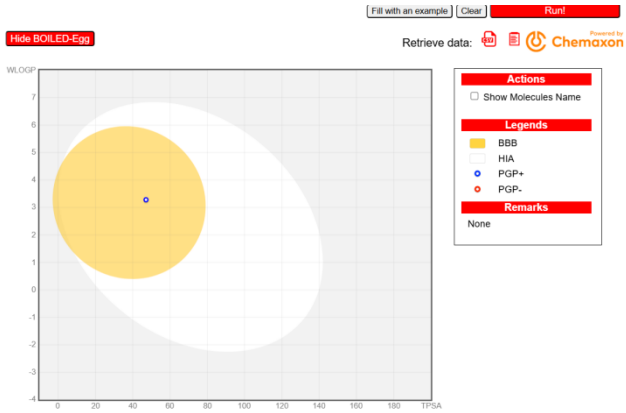


Figure 16. BOILED-Egg model prediction of pharmacokinetics for Nigellidine

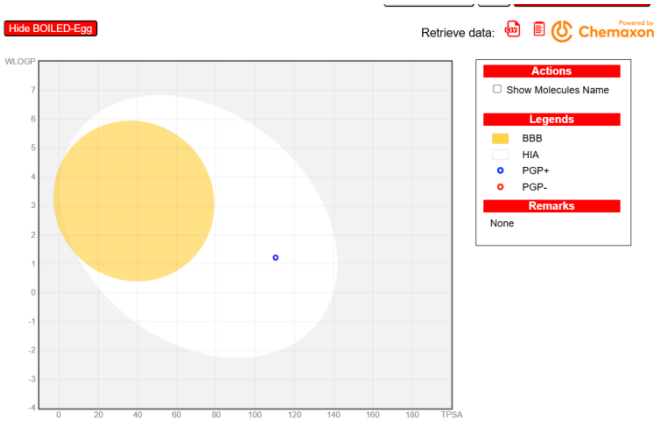


Figure 17 .BOILED-Egg model prediction of pharmacokinetics for Catechin

The analysis of Swiss ADME yielded a range of possible pharmacokinetics for each of the examined compounds; those compounds with high gastrointestinal (GI) absorption exhibited the greatest potential for oral bioavailability. The BOILED-Egg model provided confirmation of permeability predictions and also allowed for differentiation between compounds with the ability to enter the central nervous system (CNS) vs. compounds that exert their action only within the periphery. The compound with the most favorable profile of all the screen compounds was Nigellidine which exhibited high levels of GI absorption, excellent blood-brain barrier (BBB) permeability, and interaction with P-glycoprotein. These attributes indicate that Nigellidine demonstrates the potential for good absorption, distribution, and thus serves as an excellent drug candidate. The remaining compounds displayed limited gastrointestinal absorption and/or permeability making Nigellidine the lead compound among all tested.

4. Conclusion

The present study highlights the potential of Nigella sativa seed-derived phytochemicals as promising candidates for the management of cystic fibrosis through an integrated in-silico and in-vitro approach. Molecular docking studies demonstrated that several compounds exhibited strong binding interactions with

the CFTR protein, comparable to the standard drug Ivacaftor. The validation of these results further confirmed the stability and reliability of the ligand–protein interactions. Pharmacokinetic evaluation provided additional insight into the drug-likeness and bioavailability of the selected compounds. Among the analyzed phytochemicals, Nigellidine was identified as the most promising drug lead due to its favorable balance of binding affinity and pharmacokinetic properties, particularly its high gastrointestinal absorption and permeability. Furthermore, the in-vitro antioxidant analysis supported the biological significance of *Nigella sativa*, indicating its ability to neutralize free radicals. Overall, the findings suggest that *Nigella sativa* represents a valuable natural source for potential therapeutic agents in cystic fibrosis. However, further in-vivo studies and clinical validation are necessary to confirm their efficacy and safety for practical applications.

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