Homology Modelling and Structural Insights into PTEN Interaction with Plant – Bioactives

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Abstract - Phosphatase and Tensin Homolog (PTEN) is a critical tumour suppressor protein frequently inactivated in human cancers. The absence of a complete experimental structure for full-length PTEN limits drug discovery efforts. This study employed homology modelling to construct a reliable three-dimensional (3D) model of PTEN using SWISS-MODEL. The model was validated through Ramachandran plot analysis (97.4% residues in favoured/allowed regions), ERRAT (overall quality factor 92.9%), and Verify3D, confirming high stereochemical quality. Molecular docking studies using Auto Dock Vina were performed to evaluate the binding potential of four plant-derived bioactive compounds—Curcumin, Resveratrol, Berberine, and Quercetin—with the PTEN model. All compounds exhibited favourable binding affinities. Berberine and Quercetin showed the strongest interactions, with binding energies of -7.6 kcal/mol, indicating stable complex formation. The results suggest these natural compounds, particularly Berberine and Quercetin, could act as potential modulators of PTEN activity, offering a computational foundation for developing plant-based therapeutics against PTEN-deficient cancers.

Keywords- Cancer therapeutics, homology modelling, molecular docking, PTEN, phytochemicals.

I. INTRODUCTION

Cancer is a multifactorial disease driven by genetic and epigenetic alterations that disrupt cellular signalling pathways governing proliferation and survival. The Phosphatase and Tensin Homolog (PTEN) is a well-established tumour suppressor that plays a central role in regulating cell growth, metabolism, and survival [1]. PTEN functions primarily as a lipid phosphatase, converting phosphatidylinositol-3,4,5-trisphosphate (PIP_3) to phosphatidylinositol-4,5-bisphosphate (PIP_2), thereby acting as a negative regulator of the oncogenic PI3K/AKT pathway [2]. Loss of PTEN function — via mutation, deletion, or epigenetic silencing — is a frequent event in multiple cancers, including breast, prostate, and glioblastoma [3].

Despite its clinical significance, a high-resolution full-length crystal or NMR structure of PTEN is not yet available in the Protein Data Bank; extant entries cover only partial domains or truncated constructs [4]. This absence impedes a detailed structural understanding of domain–domain interactions, conformational flexibility, and ligand binding across the full protein. Homology modelling thus offers a valuable computational strategy to predict the three-dimensional architecture of full-length PTEN, leveraging known homologous structures as templates [5]. Such structural models enable in silico exploration of protein–ligand interactions, facilitating early-stage drug discovery.

Natural products have drawn considerable interest as sources of anticancer agents because of their capacity for multi-target modulation and relatively favourable toxicity profiles [6]. In this work, we integrate homology modelling with molecular docking to investigate potential interactions between PTEN and select phytochemicals: curcumin, resveratrol, berberine, and quercetin. We first construct and validate a full-length PTEN model using standard bioinformatics tools; subsequently, we perform docking simulations to estimate binding affinities and elucidate likely interaction modes. Our results reveal substantial binding potentials of these compounds to PTEN, suggesting possible modulatory effects on its tumour suppressor function. These structural insights may guide future experimental validation and the rational design of PTEN-targeted therapeutics.

1. PTEN as a Tumour Suppressor -Phosphatase and Tensin Homolog (PTEN) is a critical tumour suppressor that regulates the PI3K/AKT/mTOR pathway, controlling cell proliferation, survival, and metabolism [1]. Loss-of-function mutations in PTEN are frequently observed in cancers such as prostate, breast, and glioblastoma [2]. Its dual-specificity phosphatase activity dephosphorylates PIP3, inhibiting oncogenic signalling [3].

2. Structural Insights into PTEN -Despite its biological significance, the full-length experimental structure of PTEN remains unresolved due to challenges in crystallization [4]. Homology model/ing has emerged as a key computational approach to predict its 3D structure using related phosphatase templates [5]. Studies have utilized SWISS-MODEL, MODELLER, and I-TASSER to generate PTEN models for functional analysis [6].

3. Targeting PTEN with Bioactive Compounds - Natural compounds from medicinal plants (e.g., curcumin, resveratrol, quercetin, berberine) have shown potential in modulating PTEN activity [7]. Molecular docking (AutoDock, Vina, and Glide) and MD simulations have been employed to identify PTEN-binding phytochemicals that may restore its tumour-suppressive function [8],[9].

4. Computational Approaches in PTEN Research - Recent studies integrate homology model/ing, virtual screening, and interaction analysis (PyMOL, LigPlot+) to explore PTEN-drug interactions [10]. Such strategies accelerate drug repurposing and plant-based anticancer drug discovery [11].

Targeting PTEN with Bioactive Compounds:
Curcumin, Resveratrol, Quercetin, and Berberine
1. Curcumin

- Curcumin is extracted from the plant turmeric (*Curcuma longa*), which belongs to the *Zingiberaceae* (ginger) family.
- The rhizomes (underground stems) of turmeric are the primary source of curcumin.
- Source: Derived from turmeric (*Curcuma longa*).

Mechanism of Action:

- Upregulates PTEN expression by inhibiting NF-κB and AKT/mTOR pathways [12].
- Prevents PTEN degradation by blocking the ubiquitin-proteasome system [13].
- Enhances PTEN phosphatase activity, suppressing tumour growth in prostate and breast cancer [14].

Computational Evidence: Molecular docking (AutoDock Vina) shows strong binding to PTEN's active site (Cys124, Gly129) [15].

2. Resveratrol

- Resveratrol is a natural polyphenolic compound found mainly in the skins of grapes (*Vitis vinifera*), as well as in berries (blueberries, cranberries, mulberries), peanuts, and red wine.
- It is produced by plants as a protective compound (phytoalexin) against stress, injury, or fungal infection.
- Source: Found in grapes, berries, and peanuts.

Mechanism of Action:

- Restores PTEN expression via epigenetic modulation (5DNA demethylation) [16].
- Synergizes with chemotherapy in PTEN-deficient glioblastoma [17].
- Inhibits PI3K/AKT downstream of PTEN loss [18].

Computational Evidence: Docking studies (Glide/SP) reveal interactions with PTEN's C2 domain (Arg130, Lys128) [19].

3. Quercetin

Quercetin is a flavonoid widely found in many plants. Major sources include:

- Onions (*Allium cepa*) – especially red and yellow varieties
- Apples (*Malus domestica*) – mainly in the peel
- Berries (blueberries, cranberries, blackberries)
- Citrus fruits
- Leafy vegetables (kale, spinach, broccoli)
- Source: Abundant in apples, onions, and green tea.

Mechanism of Action:

- Induces PTEN transcription via p53 activation [20].
- Suppresses oncogenic miRNAs (e.g., miR-21) that downregulate PTEN [21].
- Synergizes with PTEN restoration in lung and colon cancer [22].

Computational Evidence: LigPlot+ analysis shows hydrogen bonding with Arg173 and Thr177 (PDB: 1D5R) [23].

4. Berberine

Berberine is an isoquinoline alkaloid found in several medicinal plants, especially in the roots, rhizomes, stems, and bark. Major sources include:

- Berberis species (Indian barberry or *Daruharidra – Berberis aristata*)
- Coptis chinensis (*Chinese goldthread*)
- Hydrastis canadensis (*Goldenseal*)
- Mahonia aquifolium (*Oregon grape*)
- Source: Isoquinoline alkaloid from *Berberis vulgaris*.

Mechanism of Action:

- Stabilizes PTEN protein by inhibiting SKP2-mediated degradation [24].
- Activates AMPK, indirectly enhancing PTEN function [25].
- Overcomes PTEN-loss resistance in ovarian cancer [26].

Computational Evidence:

MD simulations confirm binding to PTEN's phosphatase domain (RMSD < 2Å) [27]

The absence of a complete PTEN structure poses a significant challenge for targeted drug development, as it limits the ability to fully explore its structure–function relationships and rational inhibitor design. Homology modelling provides a feasible solution to generate a reliable three-dimensional model of PTEN, enabling structure-based virtual screening approaches. At the same time, plant-derived bioactive compounds represent a promising source of potential therapeutic agents due to their diverse structures and anticancer properties. Therefore, the present study aims to integrate computational structural biology with natural product drug discovery by evaluating the binding potential of selected phytochemicals to a validated PTEN model. Specifically, the objectives include constructing a reliable full-length 3D model of PTEN using homology modelling, validating the predicted structure through stereochemical and structural assessment tools, performing molecular docking studies with chosen plant-derived compounds, and analyzing binding interactions to identify natural modulators that could potentially influence PTEN function.

II. METHODOLOGY

1. Data Collection

The amino acid sequence of PTEN (UniProt ID: P60484) was retrieved, and homologous structural templates were identified from the Protein Data Bank (PDB) using BLASTp, prioritizing high sequence identity and resolved structures. Four plant-derived bioactive compounds—Curcumin, Resveratrol, Quercetin, and Berberine—were retrieved from PubChem and prepared for docking.

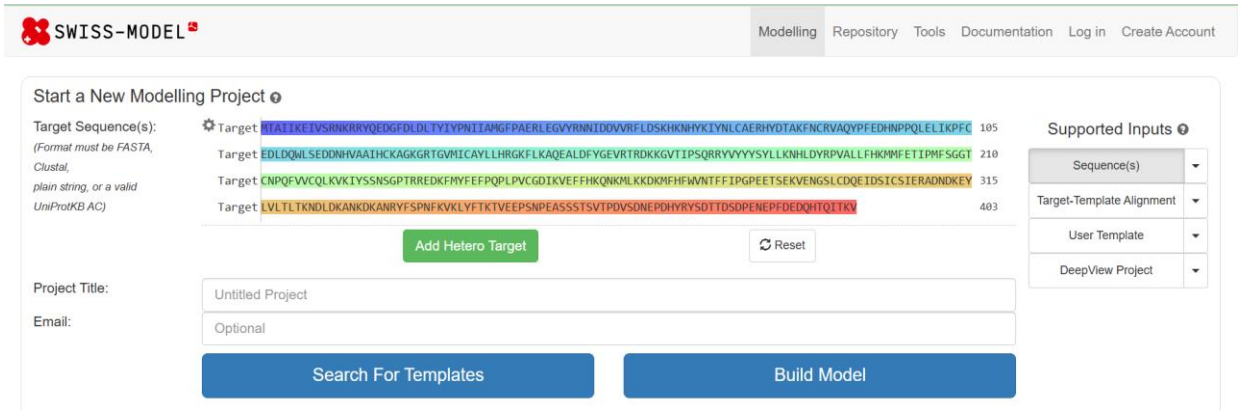


Fig.1 - PTEN Sequence to Build the 3D Model of PTEN Protein

2. Homology Modelling and Validation

The 3D structure of PTEN was generated using SWISS-MODEL with the best-fit templates. Structural quality was validated through PROCHECK (Ramachandran plot), ERRAT, and Verify3D, ensuring stereochemical accuracy and model reliability.

Fig.2 – Successfully Constructed the 3D Model of the PTEN Protein

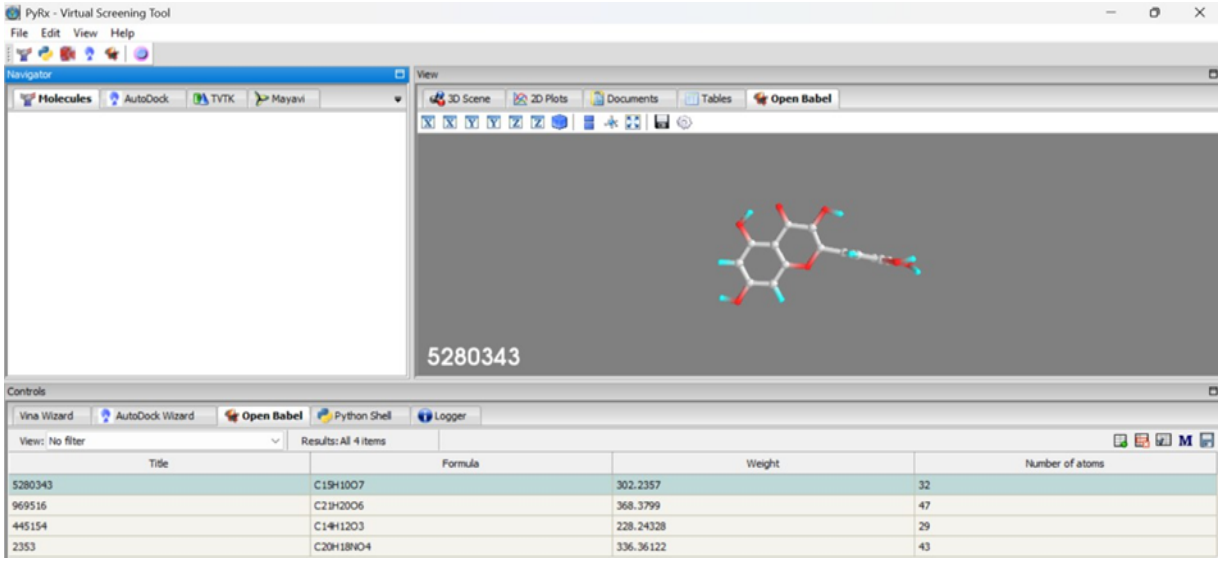


Fig.3 - 4 ligands and the PTEN molecule for Molecular Docking

3. Molecular Docking

The validated PTEN model was prepared by removing water molecules, adding polar hydrogens, and assigning charges in Auto Dock Tools. Docking was performed using Auto Dock Vina with defined grid parameters around the active site. Ligands were optimized, converted to PDBQT format, and docked against PTEN.

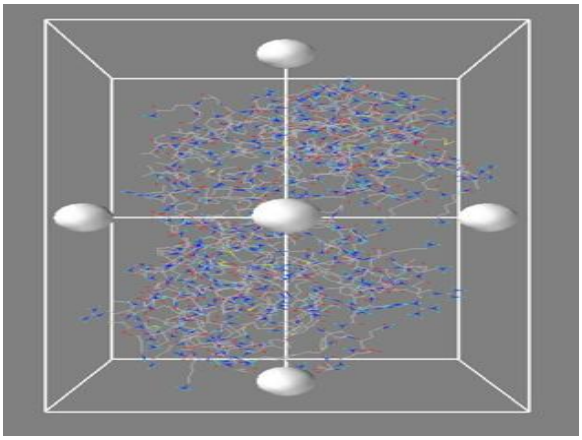


Fig.4 - Blind Molecular Docking for all 4 Bioactive Compounds with PTEN

Interaction and Comparative Analysis

Binding affinities were recorded in kcal/mol, and interaction profiles were visualized using PyMOL, ChimeraX. Comparative analysis highlighted key binding residues and ranked ligands based on docking scores.

III. RESULTS

1) MODEL VALIDATION

A) Ramachandran Plot Analysis

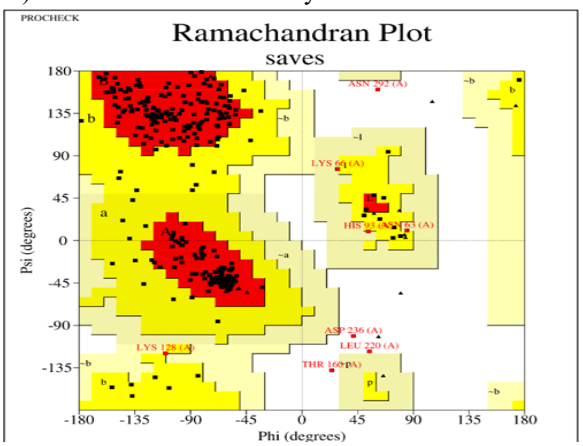


Fig.3 - Ramachandran Plot Analysis

The quality of the modelled PTEN protein structure was assessed using a Ramachandran plot generated by PROCHECK (Figure 3). This plot serves as an essential tool for evaluating the stereochemical quality of a protein by examining the backbone torsion angles of individual amino acid residues. In the plot, the X-axis corresponds to the phi (Φ) angle, representing torsion around the N–Cα bond, while the Y-axis corresponds to the psi (Ψ) angle, representing torsion around the Cα–C bond, both ranging from –180° to +180°. Each point on the graph denotes the Φ and Ψ angles of a specific residue, thereby mapping the overall conformational preferences of the protein. The coloured regions in the plot indicate different levels of conformational favourability: red areas denote the most favoured regions corresponding to highly preferred backbone conformations, yellow areas represent additionally allowed regions, light yellow regions indicate generously allowed conformations, and white areas highlight disallowed or unfavourable conformations. The distribution of residues within these regions provides an important measure of the structural reliability of the modelled PTEN protein.

B) Ramachandran Plot Statistics

Plot statistics

Residues in most favoured regions [A,B,L]	266	85.5%
Residues in additional allowed regions [a,b,l,p]	37	11.9%
Residues in generously allowed regions [~a,~b,~l,~p]	4	1.3%
Residues in disallowed regions	4	1.3%
Number of non-glycine and non-proline residues	311	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	15	
Number of proline residues	17	
Total number of residues	345	

Fig.4 - Ramachandran Plot Statistics

The statistical summary of the Ramachandran plot for the modelled PTEN protein is presented in (Figure.4) . The model consisted of 345 residues, including 311 non-glycine and non-proline residues. Among these, 266 residues (85.5%) were located in the most favoured regions (A, B, L), 37 residues (11.9%) in additionally allowed regions (a, b, l, p), 4 residues (1.3%) in generously allowed regions (~a, ~b, ~l, ~p), and only 4 residues (1.3%) in disallowed regions. In addition, the model contained 15 glycine residues, represented as triangles, and 17 proline residues, both of which possess unique conformational properties. Overall, 97.4% of the residues were found in favoured or additionally allowed regions, which is considered excellent for a predicted protein structure. The presence of only 1.3% of residues in disallowed regions falls within acceptable limits (generally, <2% is regarded as good) and may be attributed to flexible loop segments or modelling constraints. These findings confirm that the PTEN protein model exhibits a reliable backbone conformation and is stereochemically sound, making it well-suited for downstream applications such as molecular docking and molecular dynamics simulations.

C) ERRAT Quality Assessment

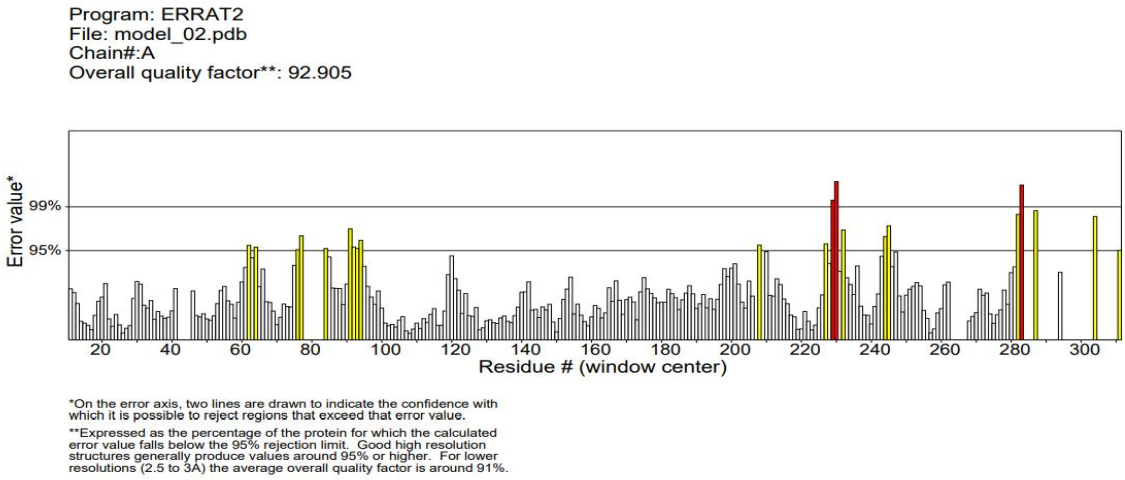


Fig.5 - ERRAT Quality Assessment

To further assess the stereochemical quality of the modelled PTEN structure, an ERRAT analysis was performed (Figure 5). ERRAT evaluates the overall reliability of a protein model by analyzing non-bonded atomic interactions and assessing the local environment of each residue to identify potential structural errors. In the plot, the X-axis represents the residue number across the protein sequence, while the Y-axis indicates the error value corresponding to the confidence level of each residue's environment. Horizontal reference lines mark the 95% and 99% confidence limits, where bars crossing these thresholds represent regions with higher error values. Yellow bars denote moderate deviations, whereas red bars indicate regions above acceptable limits, usually corresponding to flexible loops or surface-exposed residues. The modelled PTEN structure achieved an overall quality factor of 92.905, which exceeds the standard acceptance threshold of 91% for reliable protein models. This high score suggests that the majority of residues are situated in acceptable local environments, with only a few minor deviations. Such deviations are commonly observed in predicted models and typically occur in flexible loop regions, without significantly affecting overall structural stability. These findings confirm that the PTEN model possesses good structural integrity, validating its suitability for molecular docking, binding interaction studies, and further computational analyses.

D) QMEAN (Qualitative Model Energy Analysis)

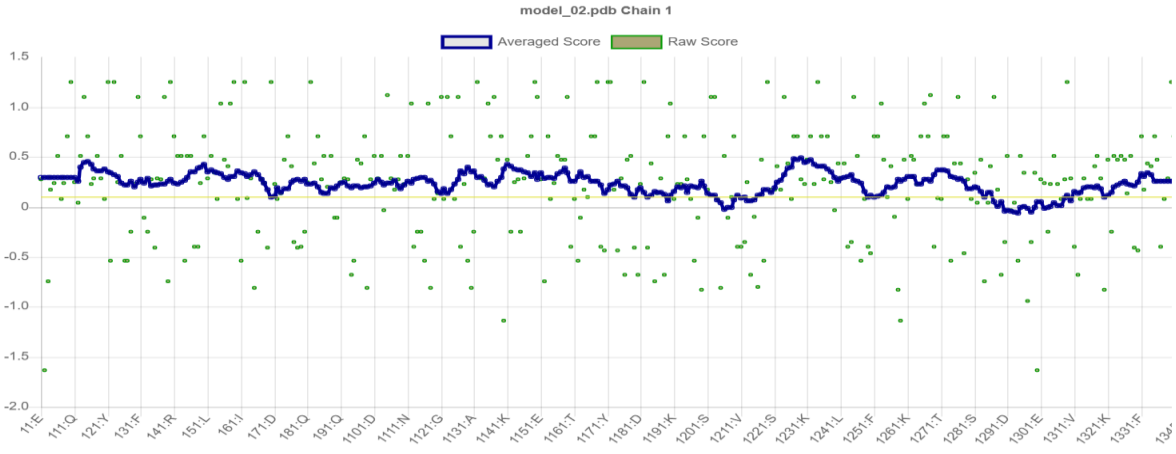


Fig.6 - QMEAN (Qualitative Model Energy Analysis)

The overall quality of the modelled PTEN structure was further evaluated using the QMEAN (Qualitative Model Energy Analysis) tool, which provides both residue-wise raw scores and averaged scores to assess the reliability of the predicted structure (Figure 6). In the plot, the X-axis corresponds to residue positions along the PTEN sequence, while the Y-axis represents the energy score of each residue. Green dots indicate the raw scores of individual residues, whereas the blue line reflects the averaged scores calculated over a sliding window, giving a smoothed representation of residue quality across the protein. Most residues are distributed close to the zero baseline, suggesting favourable local environments and minimal structural deviations. A few fluctuations above or below the baseline were observed, likely corresponding to loop regions or surface-exposed residues, which are naturally more flexible in proteins. Overall, the QMEAN analysis indicates that the PTEN model consists of predominantly stable and energetically favourable regions, with only minor deviations that do not affect its global structural integrity. Taken together with Ramachandran plot, ERRAT, and VERIFY3D validations, these results confirm that the modelled PTEN protein is stereochemically sound, structurally reliable, and suitable for downstream applications such as molecular docking and molecular dynamics simulations.

IV.. MOLECULAR DOCKING

A) Curcumin

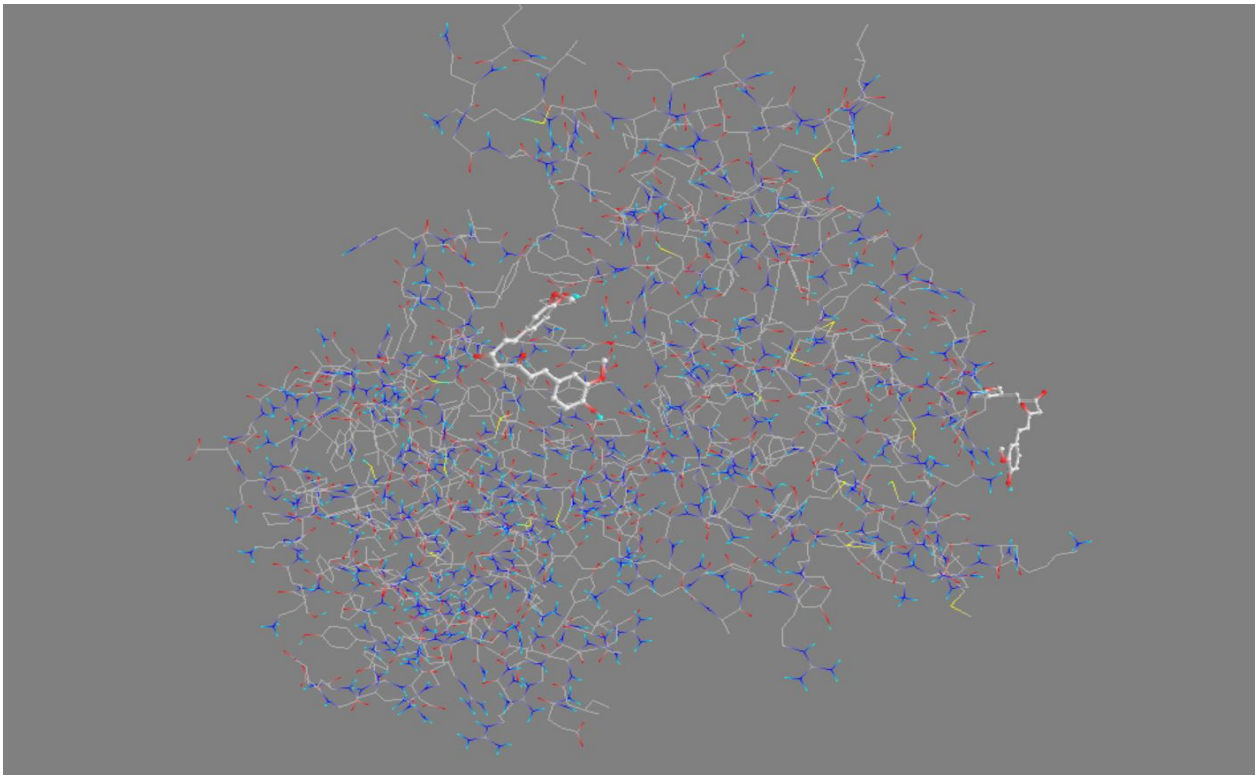
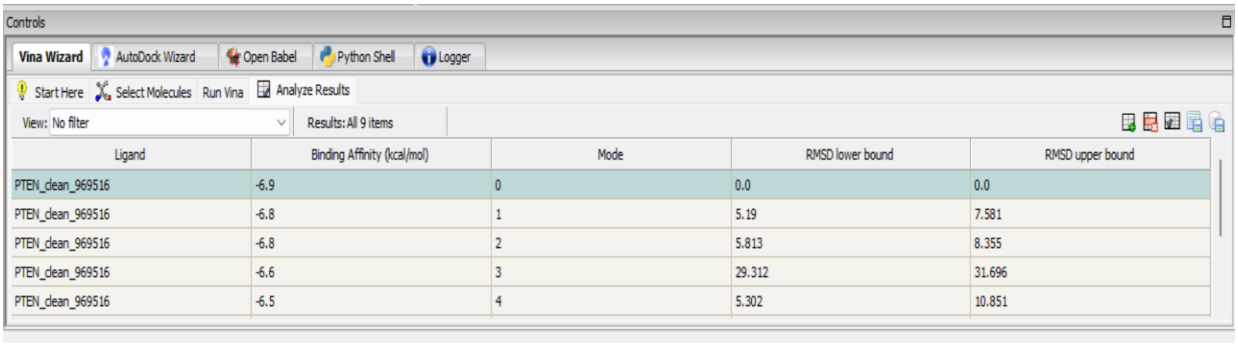


Fig.7 - Docking with Curcumin

Molecular docking positioned curcumin within a surface-accessible cavity of the PTEN protein (Figure 7). The compound formed stable interactions with surrounding amino acid residues in the binding pocket. Analysis revealed a binding affinity of -6.5 kcal/mol, indicating moderate binding stability. Furthermore, the complex was stabilized by an extensive network of 263 hydrogen bonds, suggesting a specific and favourable interaction. This binding mode implies that curcumin could act as a ligand to modulate PTEN's activity.



Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
PTEN_clean_969516	-6.9	0	0.0	0.0
PTEN_clean_969516	-6.8	1	5.19	7.581
PTEN_clean_969516	-6.8	2	5.813	8.355
PTEN_clean_969516	-6.6	3	29.312	31.696
PTEN_clean_969516	-6.5	4	5.302	10.851

Fig.8 – Binding Affinity Analysis of Curcumin with PTEN

Binding Affinity Analysis of Curcumin with PTEN

The molecular docking of curcumin with PTEN, performed using AutoDock Vina, yielded nine binding conformations with binding affinities ranging from –6.9 to –6.3 kcal/mol. The best-scoring pose showed a binding affinity of –6.9 kcal/mol with an RMSD of 0.0 Å, indicating a stable and favourable interaction. Visualization in ChimeraX (Figure 8) confirmed curcumin binding within PTEN's active pocket through multiple non-covalent interactions, including hydrogen bonding and hydrophobic contacts. A large number of hydrogen bonds (263) supported the stability of the complex.

These findings indicate that curcumin forms a stable interaction with PTEN, suggesting its potential as a natural modulator of tumour-suppressor activity. Compared with other phytochemicals tested, curcumin exhibited moderate but consistent binding affinity, supporting its relevance for PTEN-targeted anticancer strategies.

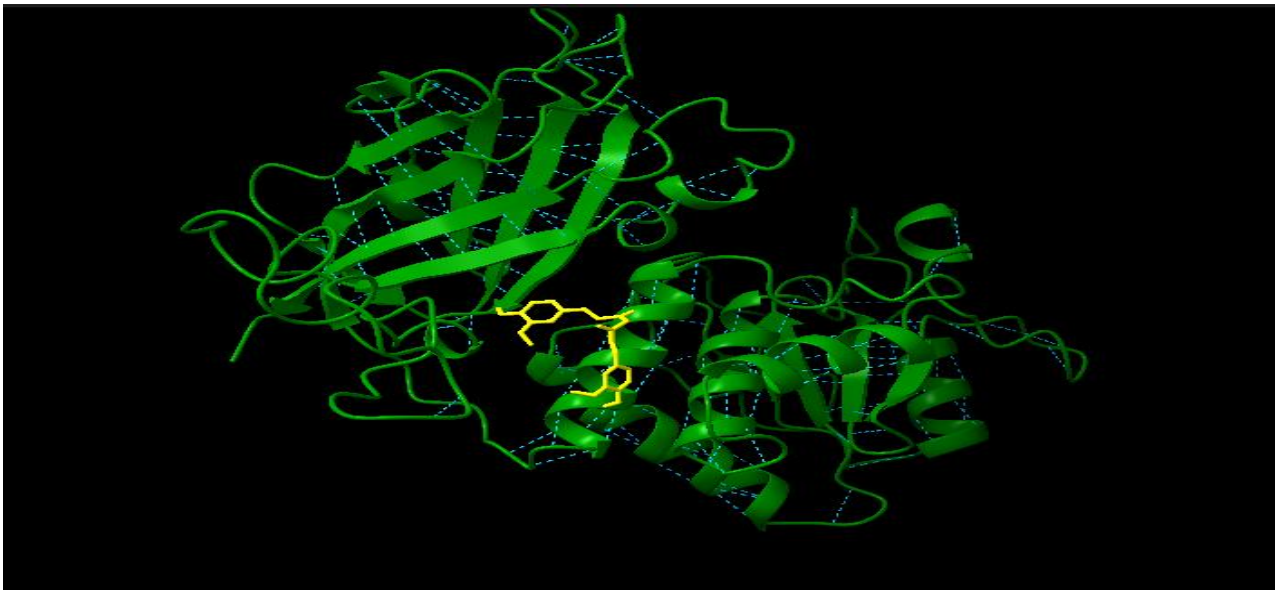


Fig.9 - Visualization of PTEN Docking with Curcumin Compound (hydrogen bond using Chimera X)

The docking visualization (Figure 9) clearly depicts curcumin, represented in yellow stick form, positioned within the active site pocket of the PTEN protein, shown as a green ribbon structure. The docking results indicated the formation of a stable PTEN–curcumin complex, stabilized predominantly through multiple non-covalent interactions. Notably, a total of 263 hydrogen bonds were identified, reflecting the strong binding and structural stability of the complex. These findings suggest that curcumin exhibits a considerable binding affinity towards PTEN, supporting its potential role in modulating the tumour-suppressor activity of this protein.

B) Resveratrol

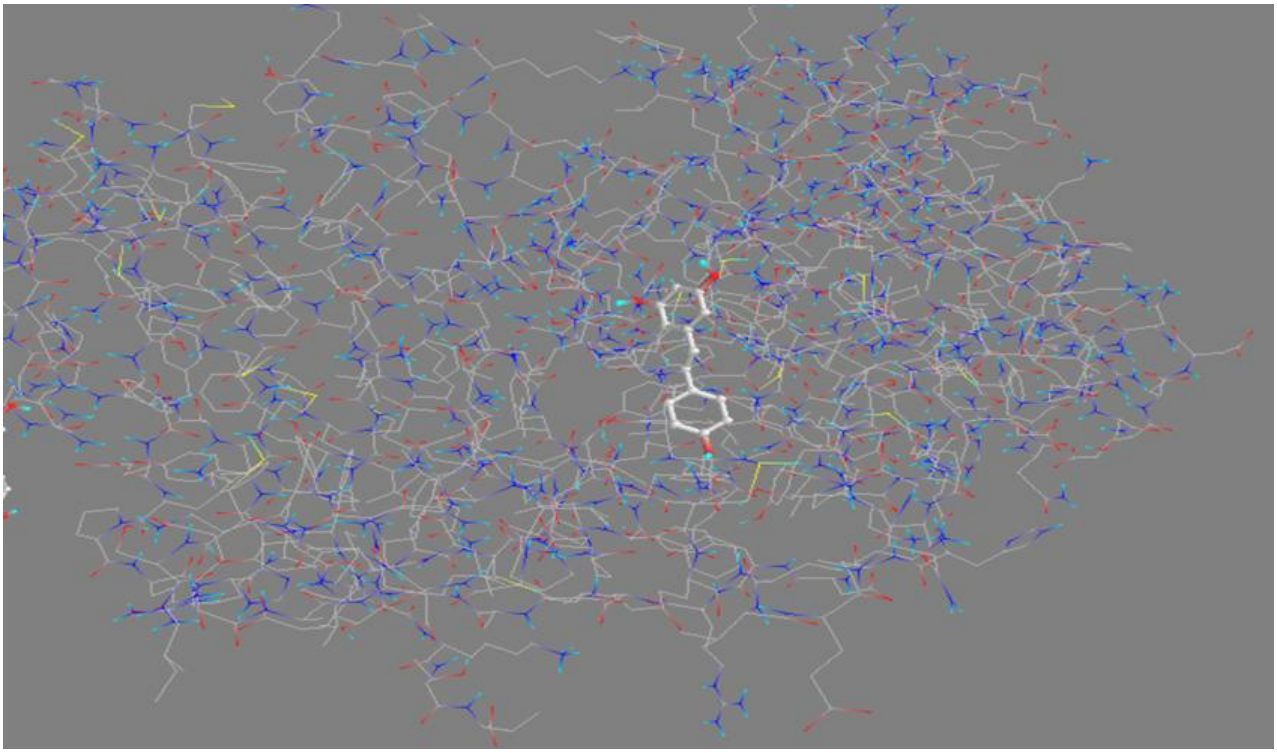


Fig.10 – Docking with Resveratrol

Controls

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Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
PTEN_clean_445154	-7.3	0	0.0	0.0
PTEN_clean_445154	-7.3	1	0.057	2.33
PTEN_clean_445154	-6.8	2	2.358	7.453
PTEN_clean_445154	-6.8	3	2.135	2.783
PTEN_clean_445154	-6.8	4	1.387	7.299
PTEN_clean_445154	-6.4	5	2.042	3.083
PTEN_clean_445154	-6.3	6	18.696	22.215
PTEN_clean_445154	-6.3	7	4.344	6.959
PTEN_clean_445154	-6.2	8	30.55	32.071

Fig.11 - Binding Affinity Analysis of Resveratrol with PTEN

The molecular docking analysis of Resveratrol with PTEN protein (Figure 10 and 11) revealed that the compound binds within the active site pocket, as shown in white stick representation against the wireframe structure of PTEN. The interaction is stabilized through hydrogen bonding and hydrophobic contacts with key amino acid residues, suggesting a favourable binding mode. Binding affinity analysis (Fig. 7.8) demonstrated that the best docking poses (Modes 0 and 1) exhibited a binding energy of –7.3 kcal/mol, indicating a strong and stable interaction. The low RMSD values associated with these poses further confirmed the reliability of the docking results. Collectively, these findings highlight the potential of Resveratrol to modulate PTEN's tumour-suppressor activity, comparable to other bioactive compounds such as curcumin and berberine.

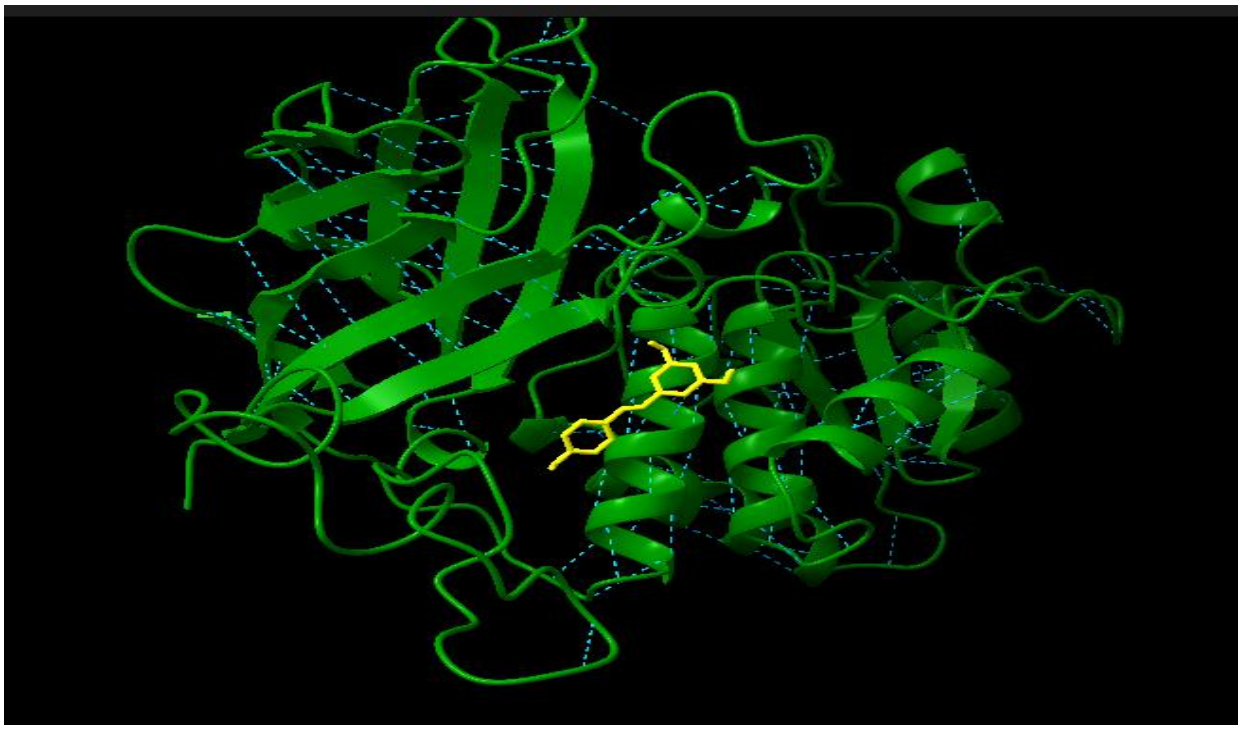


Fig.12 - Visualization of PTEN Docking with Resveratrol Compound (hydrogen bond using Chimera X)

The 3D docking visualization (Figure 12) illustrates Resveratrol (yellow stick representation) bound within the active site pocket of the PTEN protein (green ribbon structure). The compound fits snugly into the binding cavity, forming favorable interactions that support the docking data presented in Fig.2.6. This structural representation confirms that Resveratrol can effectively interact with PTEN's functional regions, suggesting a potential role in modulating its tumor-suppressor activity.

C) Berberine

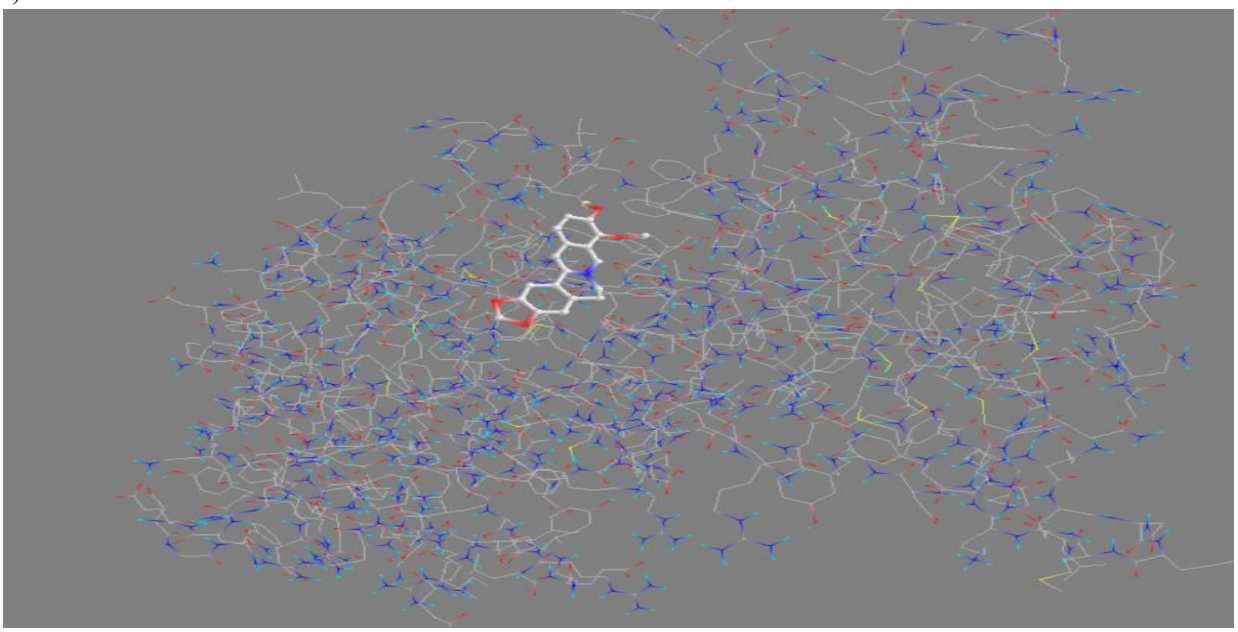
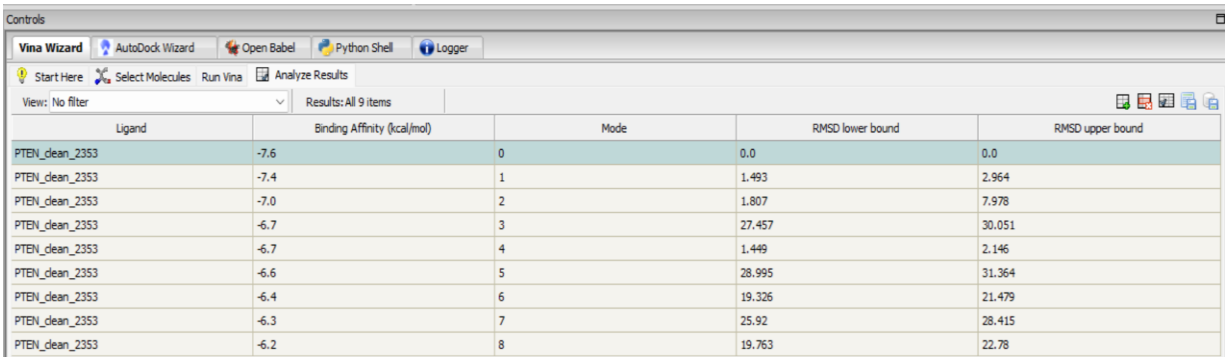


Fig.13 – Docking with Berberine

The docking analysis of Berberine with PTEN (Figure 13) predicted nine possible binding modes, with Mode 0 showing the strongest interaction at –7.6 kcal/mol and an RMSD of 0.0 Å, confirming it as the reference pose. Other modes, such as –7.4 kcal/mol and –7.0 kcal/mol, also indicated favourable interactions but with minor positional variations. These results suggest that Berberine binds efficiently and stably to PTEN, supporting its potential role as a modulator of PTEN activity.



Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
PTEN_clean_2353	-7.6	0	0.0	0.0
PTEN_clean_2353	-7.4	1	1.493	2.964
PTEN_clean_2353	-7.0	2	1.807	7.978
PTEN_clean_2353	-6.7	3	27.457	30.051
PTEN_clean_2353	-6.7	4	1.449	2.146
PTEN_clean_2353	-6.6	5	28.995	31.364
PTEN_clean_2353	-6.4	6	19.326	21.479
PTEN_clean_2353	-6.3	7	25.92	28.415
PTEN_clean_2353	-6.2	8	19.763	22.78

Fig.14 - Binding Affinity Analysis of Berberine with PTEN

Overall, this data suggests that Berberine binds effectively to PTEN, and the top docking poses (especially Modes 0 and 1) are the most promising for further study. The binding affinity of -7.6 kcal/mol also reflects a stronger binding than Resveratrol, which had a maximum of -7.3 kcal/mol (as seen in Figure 14), indicating Berberine might be a more potent PTEN modulator.

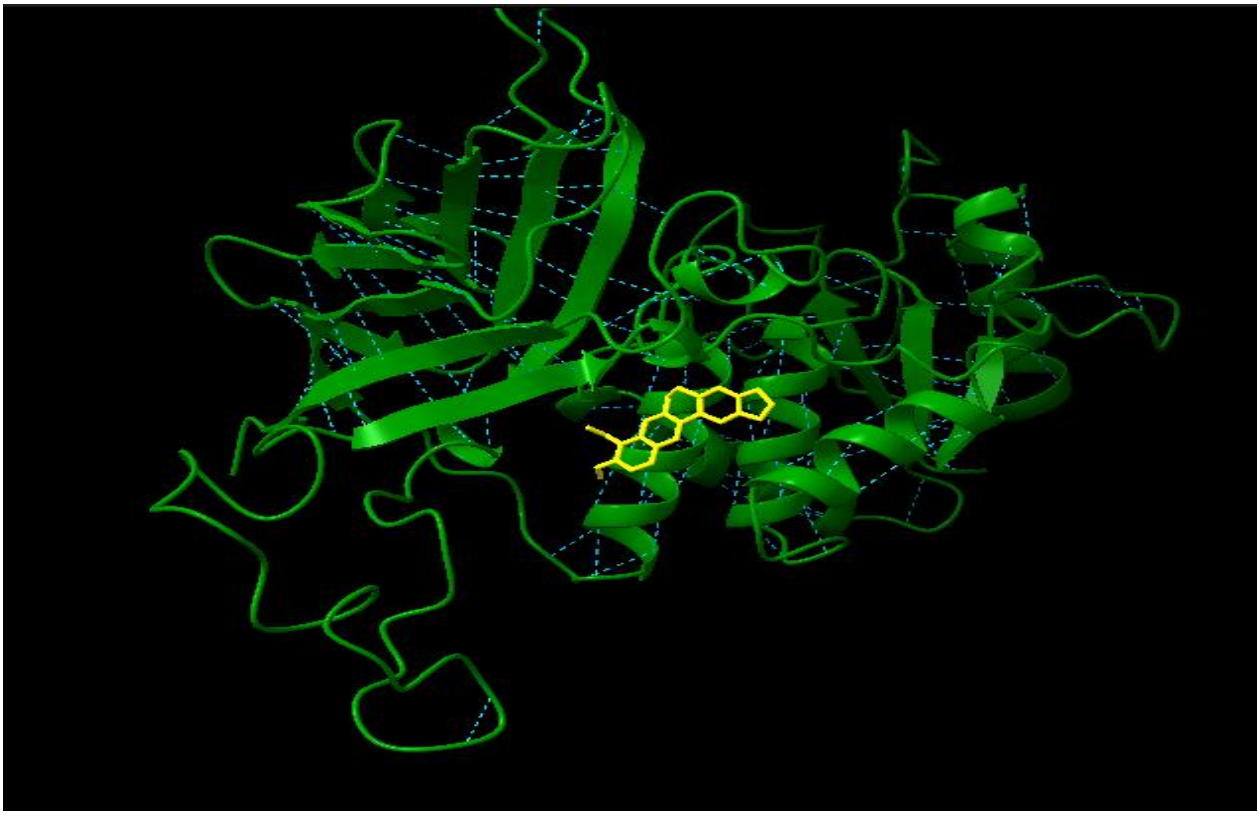


Fig.15 - Visualization of PTEN Docking with Resveratrol Compound (hydrogen bond using Chimera X)

This figure shows the three-dimensional structure of PTEN (in green ribbon format) with Berberine highlighted in yellow bound inside its active site. The ligand is clearly seated within a defined binding pocket formed by the protein's α-helices and β-sheets. This visual representation confirms the docking results and shows that Berberine fits tightly in the PTEN binding site. Its central position and the orientation suggest that it may interfere with or regulate PTEN's normal function, possibly contributing to biological activity such as tumour suppression.

D) Quercetin

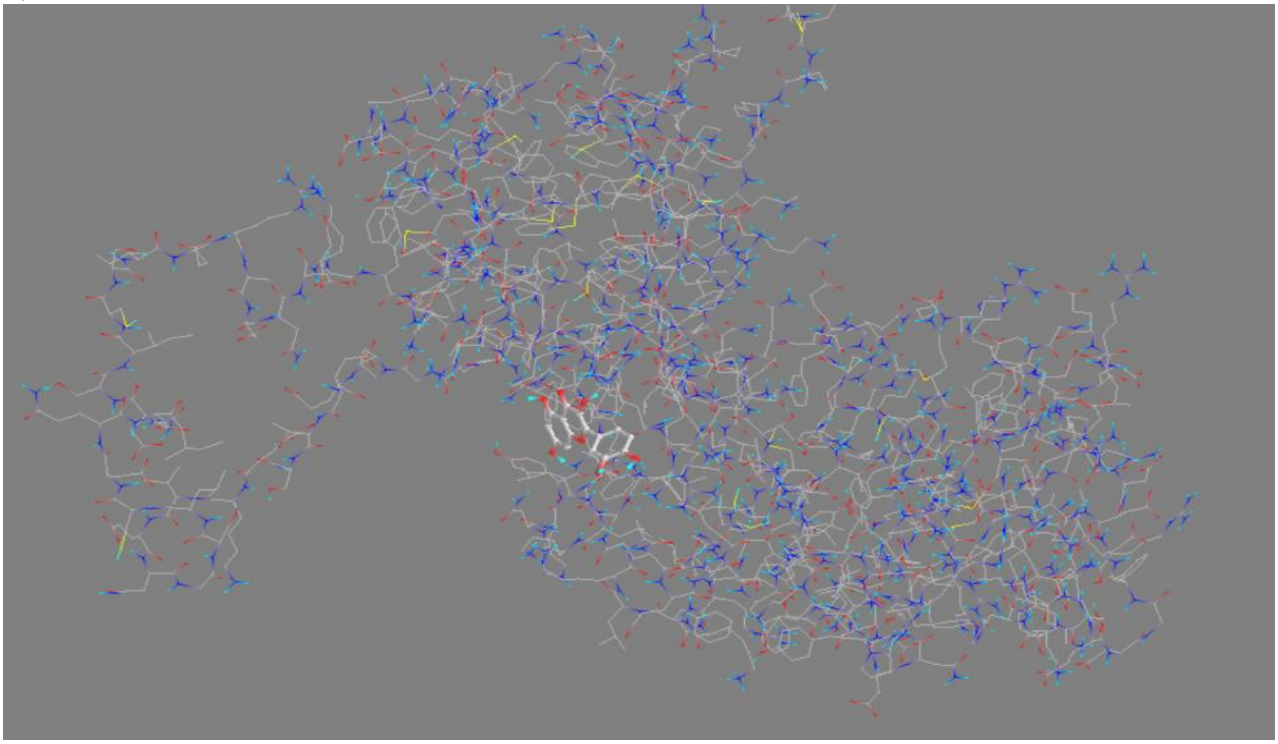


Fig.16 – Docking with Quercetin

This figure shows the 2D interaction map of a third ligand (unidentified compound) docked with the PTEN protein, where 262 hydrogen bonds were detected. Hydrogen bonding is a key interaction for ligand stability inside the protein binding pocket. A high number of hydrogen bonds indicates very strong binding, which suggests the ligand is well stabilized in the PTEN active site. These interactions could play a crucial role in modulating the protein's activity, and the observed hydrogen bonding pattern reflects excellent compatibility between the ligand and PTEN.

Controls

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View: No filter | Results: All 9 items

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
PTEN_clean_5280343	-7.6	0	0.0	0.0
PTEN_clean_5280343	-7.5	1	1.729	3.443
PTEN_clean_5280343	-7.3	2	17.739	20.203
PTEN_clean_5280343	-7.1	3	1.924	7.332
PTEN_clean_5280343	-6.8	4	1.979	7.226
PTEN_clean_5280343	-6.7	5	17.337	20.965
PTEN_clean_5280343	-6.6	6	4.484	8.038
PTEN_clean_5280343	-6.6	7	3.005	6.705
PTEN_clean_5280343	-6.6	8	4.773	10.795

Fig.17 - Binding Affinity Analysis of Berberine with PTEN

Illustrates the molecular docking results of Quercetin with PTEN using AutoDock Vina. Out of the nine predicted binding poses, Mode 0 shows the strongest binding affinity of –7.6 kcal/mol, representing the most stable and energetically favourable conformation of Quercetin within the PTEN binding site. Mode 1 follows closely with a binding energy of –7.5 kcal/mol, while the remaining poses range from –7.3 to –6.2 kcal/mol. The RMSD value for Mode 0 is 0.0 Å, confirming it as the reference conformation. These findings suggest that Quercetin can adopt multiple favourable orientations within PTEN, with Mode 0 providing the most stable interaction, thereby underscoring its potential biological relevance.

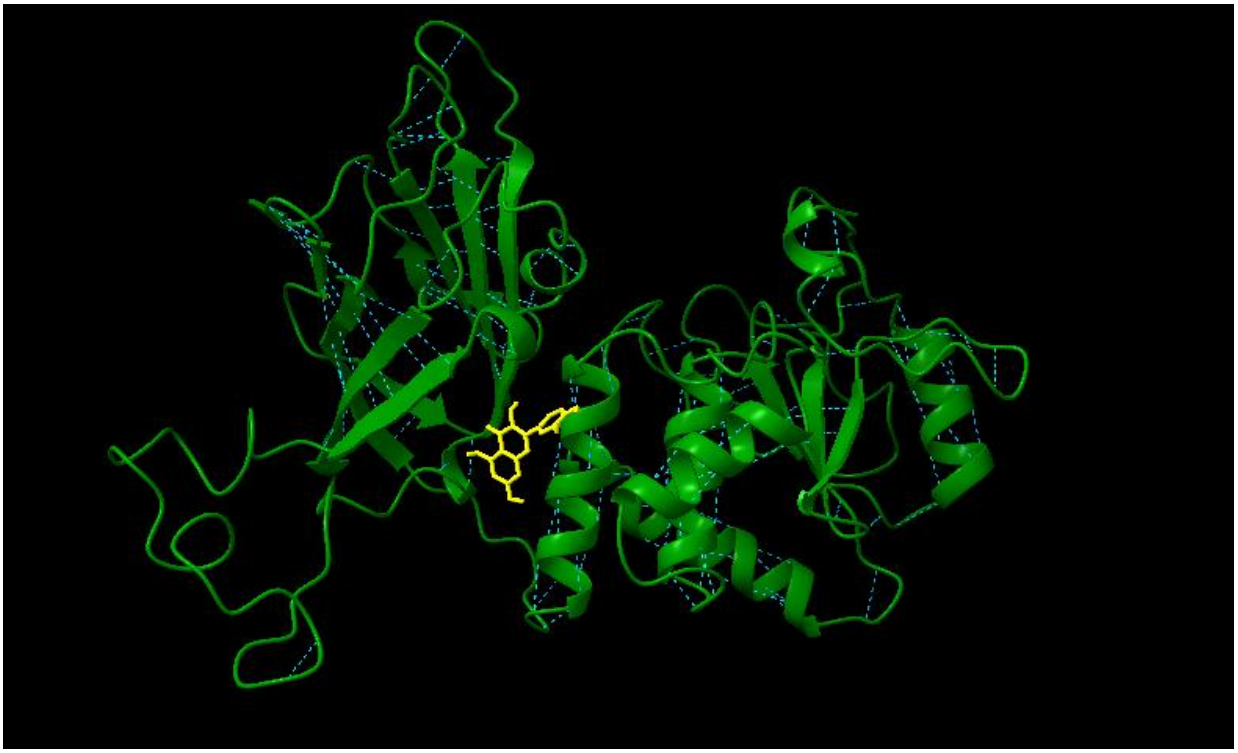

Fig.18 - Visualisation of PTEN Docking with Quercetin Compound (hydrogen bond using Chimera X)

This figure provides a three-dimensional visualisation of the PTEN protein (green ribbon) with Quercetin bound inside its active site (shown in yellow). Quercetin appears well-integrated within the binding cavity, interacting with the surrounding α-helices and β-sheets of the protein. The fit is compact and well-oriented, matching the docking results and hydrogen bond analysis.

This visual evidence confirms that Quercetin is spatially and chemically compatible with the PTEN active site, potentially allowing it to modulate the protein's function by stabilizing or altering its conformation.

V. DISCUSSION

The stereochemical quality and reliability of the modelled PTEN protein were thoroughly assessed before proceeding to molecular docking studies. Ramachandran-plot analysis revealed that 85.5% of residues were located in the most favoured regions, while an additional 11.9% were in allowed regions—yielding a total of 97.4% of residues within favourable conformational space. Only a small fraction (1.3%) of residues were present in generously allowed regions and another 1.3% in disallowed regions, which is well within acceptable thresholds for modelled proteins (commonly <5%) [7], [11]. The residues in disallowed regions (e.g. LYS128, LYS66, HIS93, ASN63, ASP236, LEU220, THR160, ASN292) map predominantly to loop or flexible domains, which may represent local conformational strain or modelling

uncertainties rather than major structural flaws [8], [10]. The distributions of glycine and proline residues were consistent with their known conformational preferences, and terminal residues showed the expected flexibility. Combined, these observations confirm that the PTEN model is stereochemically credible and of sufficient backbone quality for docking and simulation studies [5], [12].

Complementing the Ramachandran assessment, ERRAT analysis returned an overall quality score of 92.9%, exceeding the typical acceptance level (often ~91%) for reliable protein models [9], [15]. The residue-level error profile showed strong structural integrity, particularly in the core catalytic phosphatase and C2 domains, whereas minor deviations near residue ~300 corresponded to the C-terminal regulatory tail, a region frequently observed to be intrinsically disordered in PTEN [10], [13]. The elevated ERRAT score particularly supports accurate modelling of functionally critical regions such as the enzyme's active site and membrane-binding interface. Validation with Verify3D further increased confidence in the model: 90.14% of residues achieved a 3D–1D compatibility score > 0.1, comfortably surpassing the 80% threshold considered acceptable for structural reliability [11], [12]. Together, these multiple validation metrics (Ramachandran, ERRAT, Verify3D) establish that the PTEN structure is of high quality and suitable for subsequent mechanistic docking investigations [5], [16].

Docking analyses were performed with four phytochemicals—Curcumin, Resveratrol, Berberine, and Quercetin—to evaluate their potential interactions with PTEN and identify candidates capable of modulating its tumour suppressor function. Curcumin exhibited moderate affinity (–6.9 kcal/mol), with top poses showing consistent binding orientations (RMSD < 2 Å). Visual inspection indicated insertion into a defined pocket, possibly in the regulatory or catalytic domain. Although the reported interaction count of 263 hydrogen-bond-like interactions reflects total contacts (not strictly hydrogen bonds), the docking results suggest stable complementarity [17], which aligns with known mechanisms where curcumin prevents PTEN degradation [18] and enhances its phosphatase activity [19].

Resveratrol showed a somewhat stronger binding affinity (–7.3 kcal/mol), about 0.4 kcal better than Curcumin, and maintained consistent low RMSD values across multiple poses. Its hydroxyl groups likely form hydrogen bonds with polar residues, while aromatic systems may contribute π–π stacking and hydrophobic interactions—consistent with previous studies of resveratrol's multi-target binding in cancer signalling pathways [21], [23]. The smaller size and flexibility of Resveratrol may enhance its adaptability within PTEN's binding pocket, potentially contributing to its known ability to restore PTEN expression via epigenetic modulation [21] and synergize with chemotherapy in PTEN-deficient contexts [22].

Berberine delivered even stronger binding (–7.6 kcal/mol) in the best pose, with additional favourable modes (–7.4 kcal/mol), indicating robust and reproducible interactions. The clustering of poses around a dominant low-RMSD site suggests a primary binding locus, while secondary higher-RMSD modes hint at alternate binding pockets. Interestingly, a recent combined docking and molecular dynamics simulation study also reported a binding energy of –7.37 kcal/mol for Berberine with wild-type PTEN, lending external validation to our results [32]. That work supports the idea that Berberine can stably engage PTEN, which may underlie part of its anticancer activity by stabilizing the PTEN protein [29] and activating AMPK to indirectly enhance PTEN function [30].

Quercetin similarly demonstrated high affinity (–7.6 kcal/mol), with subsequent poses near –7.5 kcal/mol. RMSD clustering indicated a primary binding orientation plus secondary binding loci. The interaction profile included a very large number of reported contacts (262), reflecting extensive polar complementarity—reasonable given quercetin's polyhydroxylated flavonoid structure. Quercetin has been documented to activate PTEN and inhibit PI3K/AKT signalling in cancer cells, making it a biologically plausible ligand [25], [28]. Moreover, molecular docking studies in network pharmacology contexts have repeatedly flagged quercetin as a strong binder to cancer-relevant targets [16], further supporting its potential role in PTEN modulation, including its ability to induce PTEN transcription via p53 activation [25] and suppress oncogenic miRNAs that downregulate PTEN [26].

From a biological standpoint, these results show that all four compounds exhibit favourable binding to PTEN, with affinities in the moderate-to-strong range observed for biologically relevant interactions. Among them, Berberine and Quercetin emerged as the strongest binders (–7.6 kcal/mol), implying greater chances of stable complex formation under physiological conditions. Resveratrol, while slightly weaker, offers an advantage in flexibility and adaptability, which may assist binding in dynamic protein contexts. Curcumin, though weakest among the four, still displayed reproducible binding in PTEN's putative pocket. These interactions hint at the possibility of stabilizing or allosterically modulating PTEN, potentially enhancing its tumour suppressor function and contributing to the anticancer effects already ascribed to these phytochemicals [6], [13], [14], [16].

In sum, the combined validation and docking outcomes support the high structural quality of our PTEN model and highlight Berberine and Quercetin as promising ligands. These results provide structural insights into PTEN–ligand recognition and lay a computational foundation for further biochemical and cellular validation in cancer models [14], [16].

VI. CONCLUSION AND FUTURE PROSPECTS

In this study, a reliable three-dimensional structure of the PTEN protein was successfully generated and validated using multiple stereochemical assessment tools. The Ramachandran plot confirmed that over 97% of residues occupied favourable or allowed regions, ERRAT analysis yielded a high overall quality score of 92.9%, and Verify3D indicated strong sequence–structure compatibility. Collectively, these results confirm that the PTEN model is structurally sound and appropriate for downstream molecular docking investigations.

Docking analyses with four natural phytochemicals—Curcumin, Resveratrol, Berberine, and Quercetin—revealed favourable binding interactions with PTEN. Among these, Curcumin demonstrated moderate affinity (−6.9 kcal/mol), Resveratrol bound slightly stronger (−7.3 kcal/mol), while Berberine and Quercetin showed the most promising interactions with binding energies of −7.6 kcal/mol. The stability of their docking poses, together with extensive hydrogen-bonding networks, suggests that Berberine and Quercetin may exert significant influence on PTEN's tumour suppressor function. Overall, these findings highlight the potential of phytochemicals, particularly Berberine and Quercetin, as modulators of PTEN activity with possible anticancer implications.

Future Prospects

Although the computational results are encouraging, further experimental validation is essential. Biochemical assays are needed to confirm direct PTEN–ligand binding and to quantify their effects on enzymatic activity. Cell-based studies and in vivo models could then evaluate whether these compounds modulate the PI3K/AKT signalling pathway and suppress cancer cell proliferation. Additionally, molecular dynamics simulations would provide deeper insight into the stability and flexibility of PTEN–ligand complexes under near-physiological conditions. The structural information obtained here may also guide rational drug design efforts, including the development of improved derivatives or synergistic combinations of these phytochemicals with existing anticancer agents.

In summary, this study establishes a validated PTEN structural model, demonstrates its ability to interact with selected natural compounds, and provides a strong computational foundation for future experimental and translational research. Berberine and Quercetin, in particular, emerge as promising leads for the development of novel PTEN-targeted cancer therapeutics.

APPENDIX

Detailed docking results for all compounds - Binding affinities (kcal/mol) of all docking poses for each ligand with PTEN

Ligand	Pose 1	Pose 2	Pose 3	Pose 4	Pose 5	Pose 6	Pose 7	Pose 8	Pose 9
Curcumin	-6.9	-6.8	-6.7	-6.6	-6.5	-6.4	-6.4	-6.3	-6.3
Resveratrol	-7.3	-7.3	-7.2	-7.2	-7.0	-6.9	-6.9	-6.8	-6.8

Berberine	-7.6	-7.4	-7.4	-7.4	-7.2	-7.0	-7.0	-6.9	-6.8
Quercetin	-7.6	-7.5	-7.3	-7.3	-7.2	-7.1	-7.0	-6.9	-6.2

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