

Arvenin I - A Potential Phytoconstituent to Target Metabolic Pathways Implicated in Type - 2 Diabetes Mellitus

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ABSTRACT

Background: *Picrorhiza kurroa* is one of the many Indian medicinal plants used for the treatment of Type 2 Diabetes Mellitus. It is also used in other herbal formulations and is indicated as an insulin modulator.

Objective: The focus of the study is to document and identify potential molecules to target metabolic pathways involved in DM through systems biology.

Materials and methods: A total of 55 phytoconstituents were identified and screened. Proteins associated with Type 2 Diabetes Mellitus (T2DM) and its complications were retrieved from the KEGG database. A compound–target–disease pathway network was subsequently constructed and visualized using Cytoscape software. The identified proteins targets were subjected to virtual screening against the selected lead phytoconstituents using AutoDock Vina. Followed by performing molecular dynamics simulations using GROMACS for 100 ns to evaluate the stability and dynamic behaviour of the selected protein–ligand complexes.

Results: Out of 55 phytoconstituents, 31 showed drug-likeness and a nontoxic effect. Further, these phytoconstituents modulated five molecular pathways associated with Type 2 Diabetes Mellitus. Gene-enrichment analysis showed that HSP90AA1, PRKCA, and TNF were highly modulated. Arvenin I and II scored the highest edge count among all the phytoconstituents by modulating vascular smooth muscle contraction, insulin, and pancreatic secretion signaling pathways. Moreover, during a 100 ns molecular simulation run, Arvenin I exhibited a stable and maximum number of intermolecular interactions with the least binding energy score with HSP90AA1 (-7.3 kcal/mol), PRKCA (-7.5 kcal/mol), and TNF (-7.3 kcal/mol) compared to the respective standard inhibitors.

Conclusion: A phytoconstituent of *P. kurroa* Arvenin I interacts effectively with the proteins HSP90AA1 and PRKCA, affecting several signaling pathways implicated in Type-2 Diabetes Mellitus. Moreover, the modulated phytoconstituents formed a stable protein complex that exhibited high affinity toward the selected proteins HSP90AA1 and PRKCA. Therefore, this can be a potential lead molecule in the management of Type-2 Diabetes Mellitus.

Keywords: Type-2 Diabetes Mellitus, *Picrorhiza kurroa*, Molecular Docking, Molecular Dynamics simulation, traditional medicine, network pharmacology

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Conflict of interest: None

1. INTRODUCTION

Diabetes affects approximately 463 million people, and the numbers are expected to rise to over 700 million by 2045, according to data from the International Diabetes Federation (IDF). Longstanding diabetes can lead to several complications, such as retinopathy, cerebrovascular disease, coronary heart disease, neuropathy, nephropathy, peripheral vascular disease, diabetic foot, sleep apnea, gingivitis, and complications in pregnancy [1].

Conventional allopathic management of diabetes relies on drug classes such as biguanides (metformin), sulfonylureas, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, thiazolidinediones, and insulin, which lower blood glucose by improving insulin sensitivity, enhancing insulin secretion, reducing hepatic glucose production, or increasing urinary glucose excretion. However, they are associated with side effects after long-term use, such as allergies and insulin resistance, as well as edema, nausea, and vomiting. They are also associated with lipodystrophy.

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lipohypertrophy, and many other clinical conditions [2,3]. Additionally, many herbal plant products are also used for diabetes treatment because the traditional system has many advantages from a health perspective. Many bioactive phytochemicals interact with and target the proteins associated with disease progression and play a crucial role in the pathogenicity of the disease [4,5,6,7,8]. Studies have shown that the extracts obtained from herbs like *Zingiber officinale*, *Gymnema sylvestre*, *Picrorhiza kurroa* (*P. kurroa*), etc. are effective for the treatment of diabetes [9]. Traditional healers and practitioners have well-documented *P. kurroa* (katuki) for the treatment of diabetes. It is native to the Himalayan region, ranging from Kashmir to Sikkim and Nepal. The roots and rhizomes are generally used as part of Indian Ayurvedic medicine to treat liver diseases, digestive ailments, and Type 2 Diabetes Mellitus (T2DM) [10,11].

Systems biology, which relies on the principle of molecular interactions, is now routinely used to identify new inhibitors or stimulators of metabolic pathways via molecular dynamics simulation, pharmacophore modeling, docking,

etc. In this study, online phytochemical databases were used for *in silico* identification of active phytoconstituents from *P. kurroa*, followed by *in silico* screening for target protein identification. The shortlisted drug candidates for diabetes management were subjected to molecular docking and MD simulations to predict the binding affinity of the phytoconstituents to the target proteins.

2. METHODOLOGY:

2.1. Identification, screening and network construction of active phytoconstituents from *P. kurroa*:

Fifty five phytoconstituents of *P. kurroa* were identified using a literature review and phytochemical databases; Dr. Dukes Phytochemical and Ethnobotanical database (<https://phytochem.nal.usda.gov>) and IMPPAT database (<https://cb.imsc.res.in/imppat/home>). The canonical SMILES for the phytoconstituents obtained from these databases with valid references were extracted from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) as represented in Supplementary Table 1.

Table 1: KEGG pathways of protein targets associated with Type-2 DM.

KEGG ID	Pathways	Protein Targets
hsa04330	Notch signaling pathway	NOTCH1, ADAM17, PSEN1
hsa04612	Antigen processing and presentation	HSPA5, HSP90AA1, TNF
hsa04911	Insulin secretion	CACNA1C, ATP1B3, ATP1A2, ATP1B1, ATP1A4, PRKCA, ATP1A3, ATP1A1
hsa04972	Pancreatic secretion	PLA2G3, ATP1B3, PRSS1, ATP2A1, ATP1A2, ATP1B1, ATP1A4, PNLIP, PLA2G2A, PRKCA, ATP1A3, ATP1A1
map04270	Vascular smooth muscle contraction	PLA2G3, CACNA1C, PRKCH, PRKCD, PLA2G2A, PRKCA

The next step involved the prediction of drug-likeness scores for the phytoconstituents retrieved using Molsoft software (<http://molsoft.com/mprop/>). With a cutoff score for drug likeness ≥ 0.06 31 phytoconstituents were shortlisted for further analysis. The toxicity profiles of the shortlisted phytoconstituents were predicted using www.way2drug.com database (Supplementary Table 2).

Table 2: Phytoconstituents and protein targets related to DM.

Phytoconstituents	Protein targets
23,24-dihydrocucurbitacin - B	PLA2G2A, PLA2G3, PRSS1, TNF
6-Feruloylcatalpol	HSP90AA1
Amphicoside	HSP90AA1
Arvenin-I	ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B1, ATP1B3, ATP2A1, HSPA5, NOTCH1, PRKCA, PSEN1
Arvenin-II	ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B1, ATP1B3, NOTCH1
Arvenin-III	ATP1A4, ATP1B1, NOTCH1
Cucurbitacin-B	ATP2A1, TNF
Cucurbitacin-F	TNF
Cucurbitacin-K	ADAM17, TNF
Cucurbitacin-O	TNF
Cucurbitacin-Q	ATP2A1
Cucurbitacin-R	ADAM17, CACNA1C, PLA2G3, TNF

Kutkoside	HSP90AA1
Minecoside	HSP90AA1
Picroside	HSP90AA1
Picroside-II	HSP90AA1
Picroside-III	HSP90AA1
Pikuroside	HSP90AA1
Tripalmitin	PNLIP, PRKCA, PRKCD, PRKCH
Verminoside	HSP90AA1, PRKCA

The next step in this analysis was identification of the protein targets for the shortlisted phytoconstituents. The data were obtained using the BindingDB database (https://www.bindingdb.org/bind/chemsearch/marvin/FMC_T.jsp), which provided information related to all the

probable protein targets for the phytoconstituents. The target proteins with a Probability of Binding value ≥ 0.7 were considered for protein target identification (Supplementary Table 3).

Table 3: Pathways related to DM and the target proteins involved in the pathway.

Pathways	Proteins
Antigen processing and presentation	HSPA5, HSP90AA1, TNF
Insulin secretion	CACNA1C, ATP1B3, ATP1A2, ATP1B1, ATP1A4, PRKCA, ATP1A3, ATP1A1
Notch signaling pathway	NOTCH1, ADAM17, PSEN1
Pancreatic secretion	PLA2G3, ATP1B3, PRSS1, ATP2A1, ATP1A2, ATP1B1, ATP1A4, PNLIP, PLA2G2A, PRKCA, ATP13, ATP1A1
Vascular smooth muscle contraction	PLA2G3, CACNA1C, PRKCH, PRKCD, PLA2G2A, PRKCA

The protein targets of the phytoconstituents were submitted to StringDB (<https://string-db.org>). This provides the interactions between the target proteins and their related disease pathways, as listed in Table 1. The protein-protein

interactions are shown in Figure 1. The pathways were analyzed with the KEGG database, and only those associated with T2DM were listed along with their protein targets.

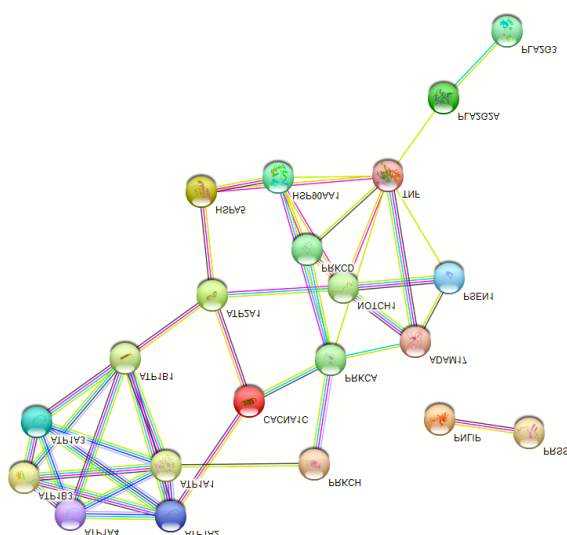


Figure 1: Protein-protein interactions of the target proteins

Figure 1: Protein-protein interactions of the target proteins. The various interactions of the proteins are represented via colour codes. The colored balls indicate the proteins and

their structure i.e., known or predicted. The edges represent protein-protein interactions – teal colour is for curated databases, purple colour is for experimentally determined,

green colour for gene neighborhood, red for gene fusion, blue for gene co-occurrence, light green for text mining, black for co-expression and light blue for protein homology.

Using Cytoscape version 3.9.1, an integrated phytoconstituent protein target KEGG pathway network was constructed to investigate the potential molecular associations of the shortlisted phytoconstituents with T2DM-related pathways and probable targets. The T2DM-specific phytoconstituent target data (Table 2) and the corresponding KEGG pathway target associations (Tables 1 2 and 3) were used for network construction, with the broader target dataset, Gene IDs and P-values (Supplementary Table 3) serving as the reference dataset.

The resulting network comprised 20 phytoconstituents, 21 protein targets and 5 KEGG pathways (46 nodes), interconnected by 50 compound–target and 32 target pathway associations (82 edges). The network was used to visualize the relationships among the shortlisted phytoconstituents, their molecular targets, and associated biological pathways, and to identify highly connected targets and pathways that may contribute to their potential effects in T2DM. The network included five T2DM-related pathways: Notch signaling, antigen processing and presentation, insulin secretion, pancreatic secretion, and vascular smooth muscle contraction. The constructed network is shown in Figure 2.

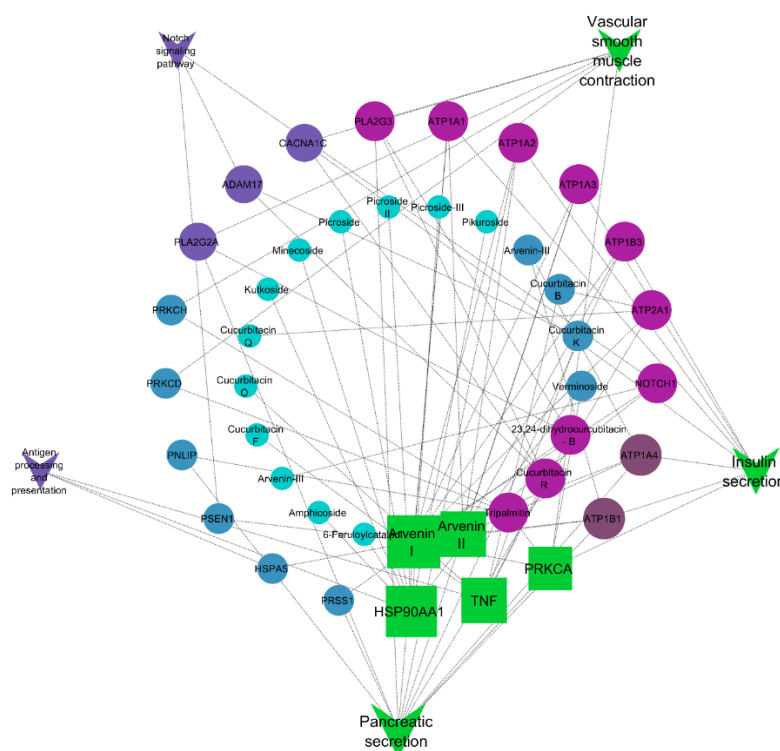


Figure 2: The network representing the interaction between the phytoconstituents, protein targets, and pathways linked to Type-2 DM (The protein targets and the phytoconstituents are represented as ball symbols, and the disease pathways are represented by arrow symbols for clarity purposes. The interactions between them are shown as lines which represent edges between nodes.)

2.2. Molecular Docking

From the network, the target proteins with the highest interaction with the phytoconstituents and the disease pathway were selected and subjected to docking analysis. The binding affinity of the phytoconstituents Arvenin I and II with proteins HSP90AA1, TNF, and PRKCA was analyzed using molecular docking based on the docking score criteria. The target protein information and structure were obtained from the UniProt database (<https://www.uniprot.org/>) and RCSB-PDB

(<https://www.rcsb.org/>), respectively. The .sdf files of the phytoconstituents and standard drugs against the protein targets were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) and the DrugBank database (<https://go.drugbank.com>). The docking procedure was conducted using the AutoDock Vina tool and visualized using Discovery Studio Visualizer (<https://discover.3ds.com/discovery-studio-visualizer-download>) (supplementary data: Figs 3 - Fig 15) [14].

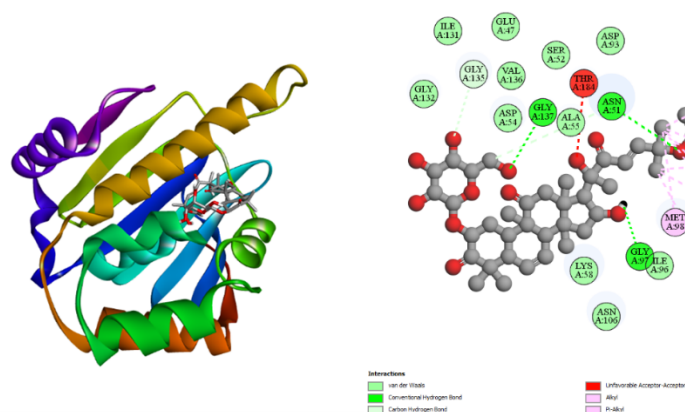


Figure 3: A) 3D representation of Arvenin I and HSP90AA1, B) 2D representation of Arvenin I and HSP90AA1 and the interacting residues of the target proteins.

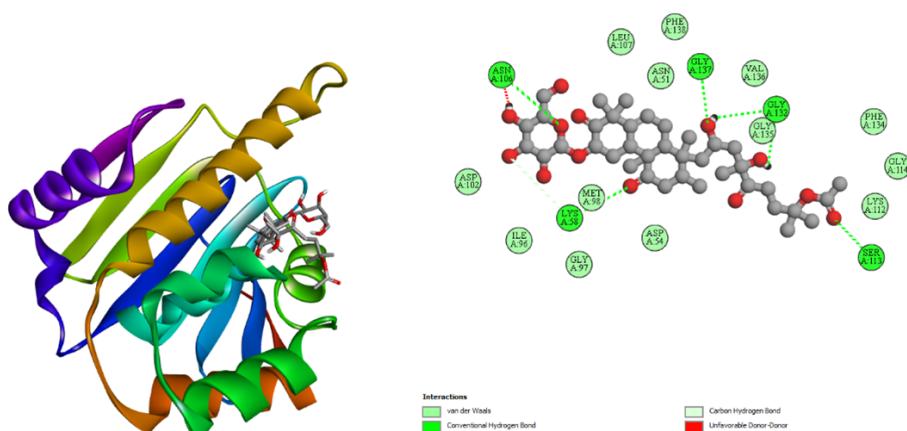


Figure 4: A) 3D representation of Arvenin II and HSP90AA1, B) 2D representation of Arvenin II and HSP90AA1 and the interacting residues of the target proteins.

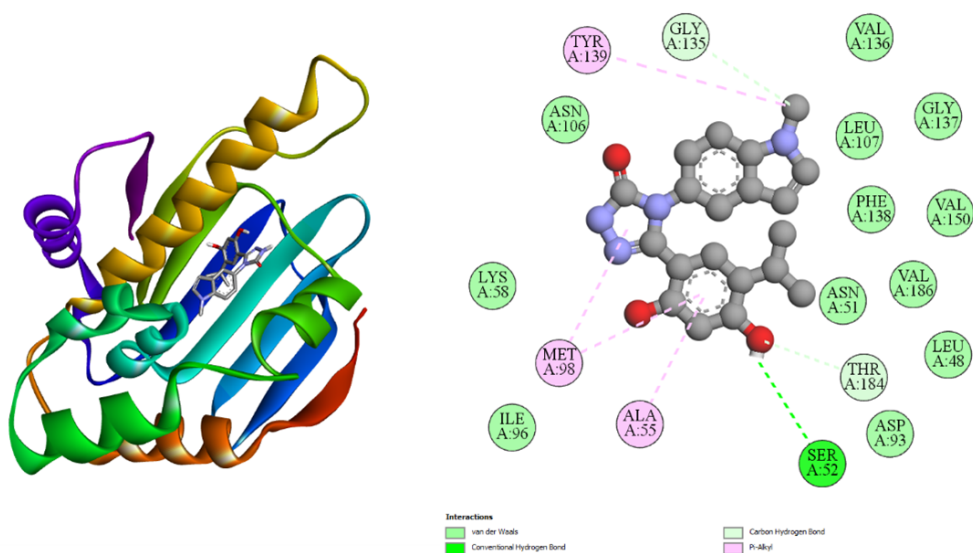


Figure 5: A) 3D representation of Ganetespi and HSP90AA1, B) 2D representation of Ganetespi and HSP90AA1 and the interacting residues of the target proteins.

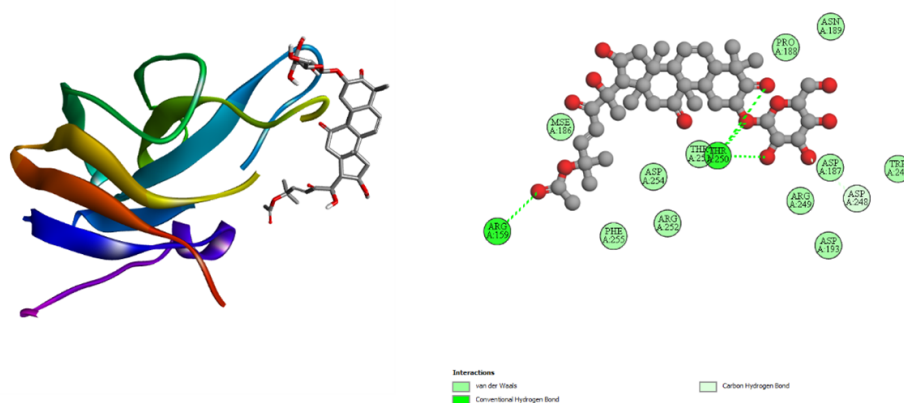


Figure 6: A) 3D representation of Arvenin I with PRKCA, B) 2D representation of Arvenin I with PRKCA and the interacting residues of the target proteins.

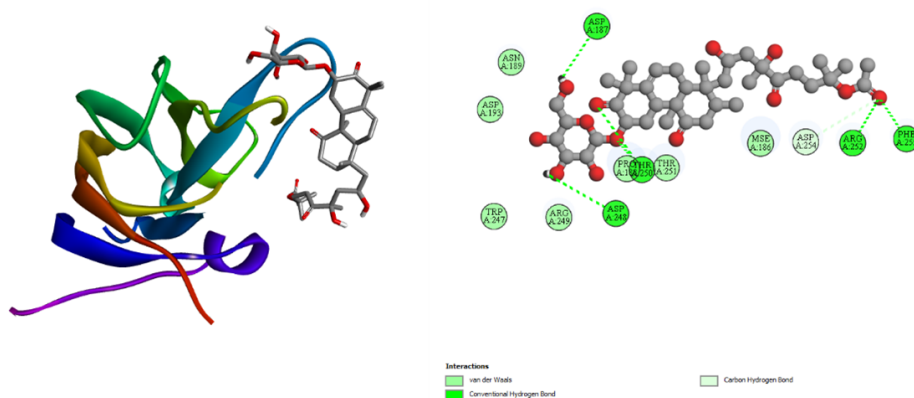


Figure 7: A) 3D representation of Arvenin II with PRKCA, B) 2D representation of Arvenin II with PRKCA and the interacting residues of the target proteins.

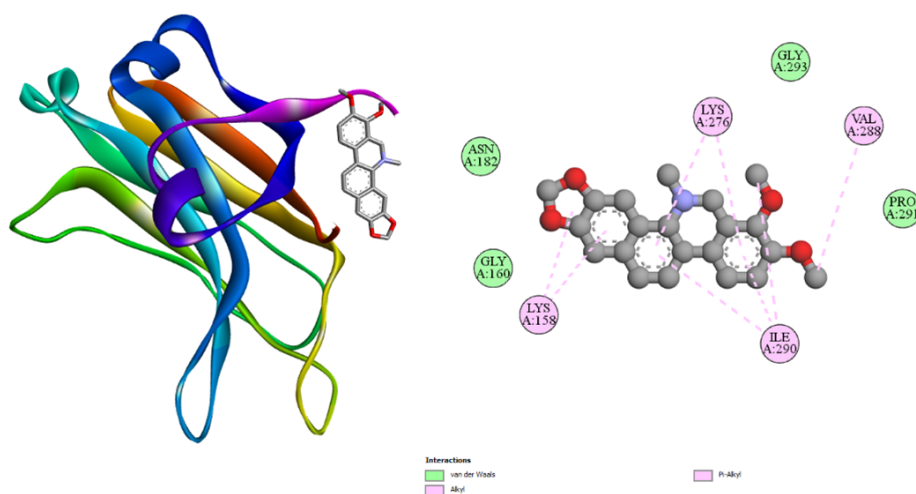


Figure 8: A) 3D representation of Chelerythrine with PRKCA, B) 2D representation of Chelerythrine with PRKCA and the interacting residues of the target proteins.

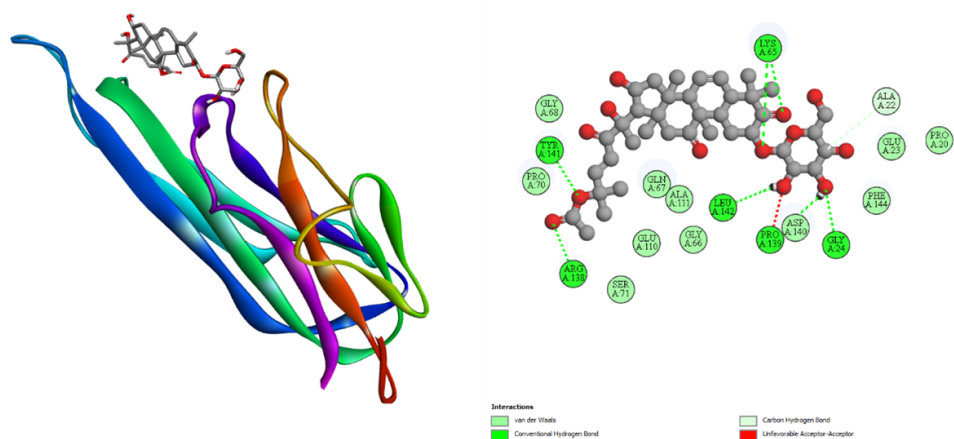


Figure 9: A) 3D representation of Arvenin I with TNF, B) 2D representation of Arvenin I with TNF and the interacting residues of the target proteins.

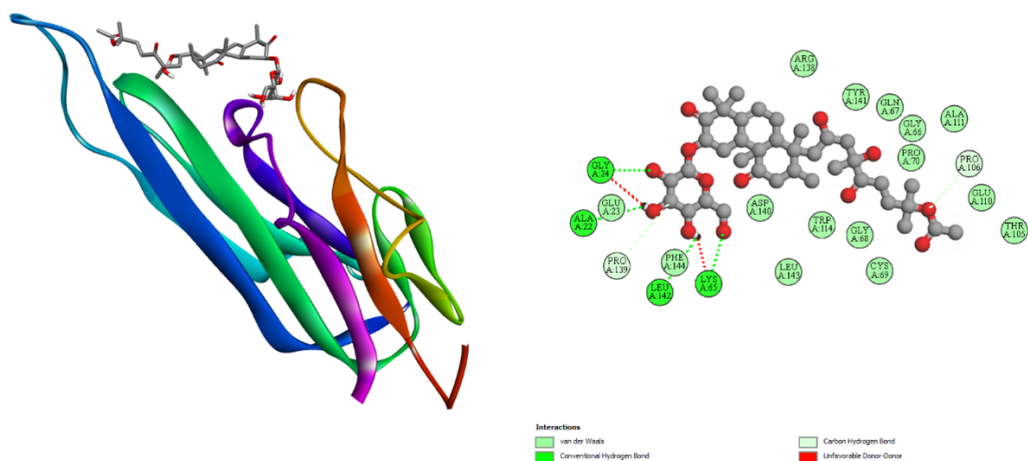


Figure 10: A) 3D representation of Arvenin II with TNF, B) 2D representation of Arvenin II with TNF and the interacting residues of the target proteins.

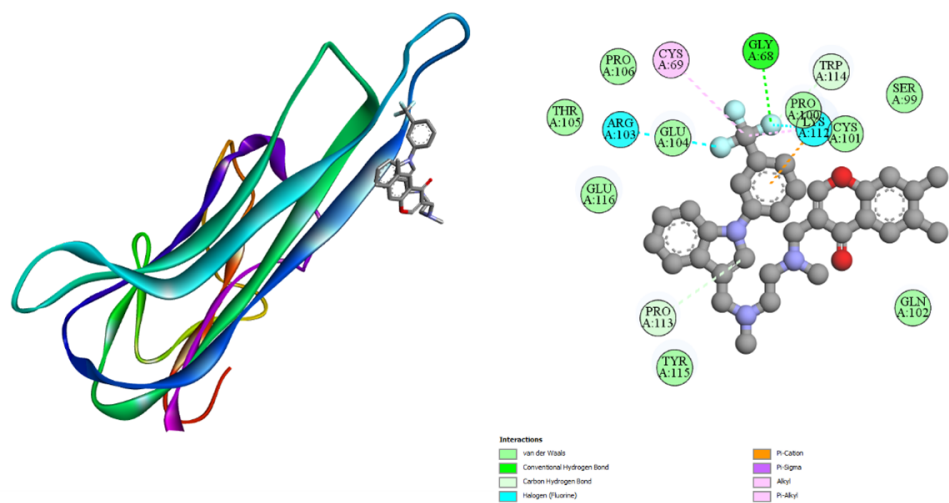


Figure 11: A) 3D representation of SPD304 with TNF, B) 2D representation of SPD304 with TNF and the interacting residues of the target proteins.

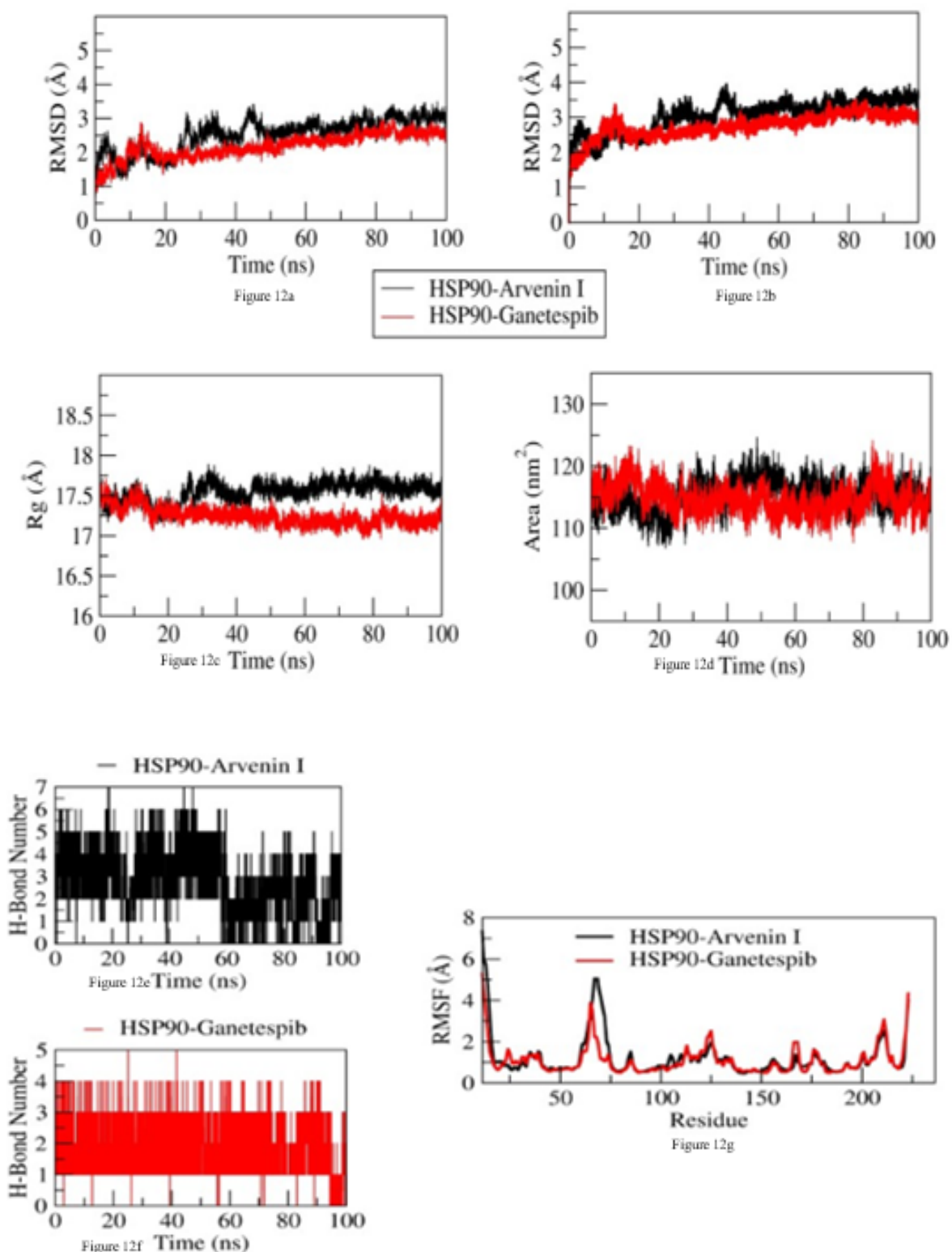


Figure 12: MD simulation analysis of complex HSP90 – Arvenin I and complex HSP90 – Ganetespiib. a) Backbone RMSD b) Complex RMSD c) Rg of the complex d) SASA plot e) Hydrogen bond between HSP90-Arvenin I f) Hydrogen bond between HSP90-Ganetespiib g) RMSF of the target protein in the complexes.

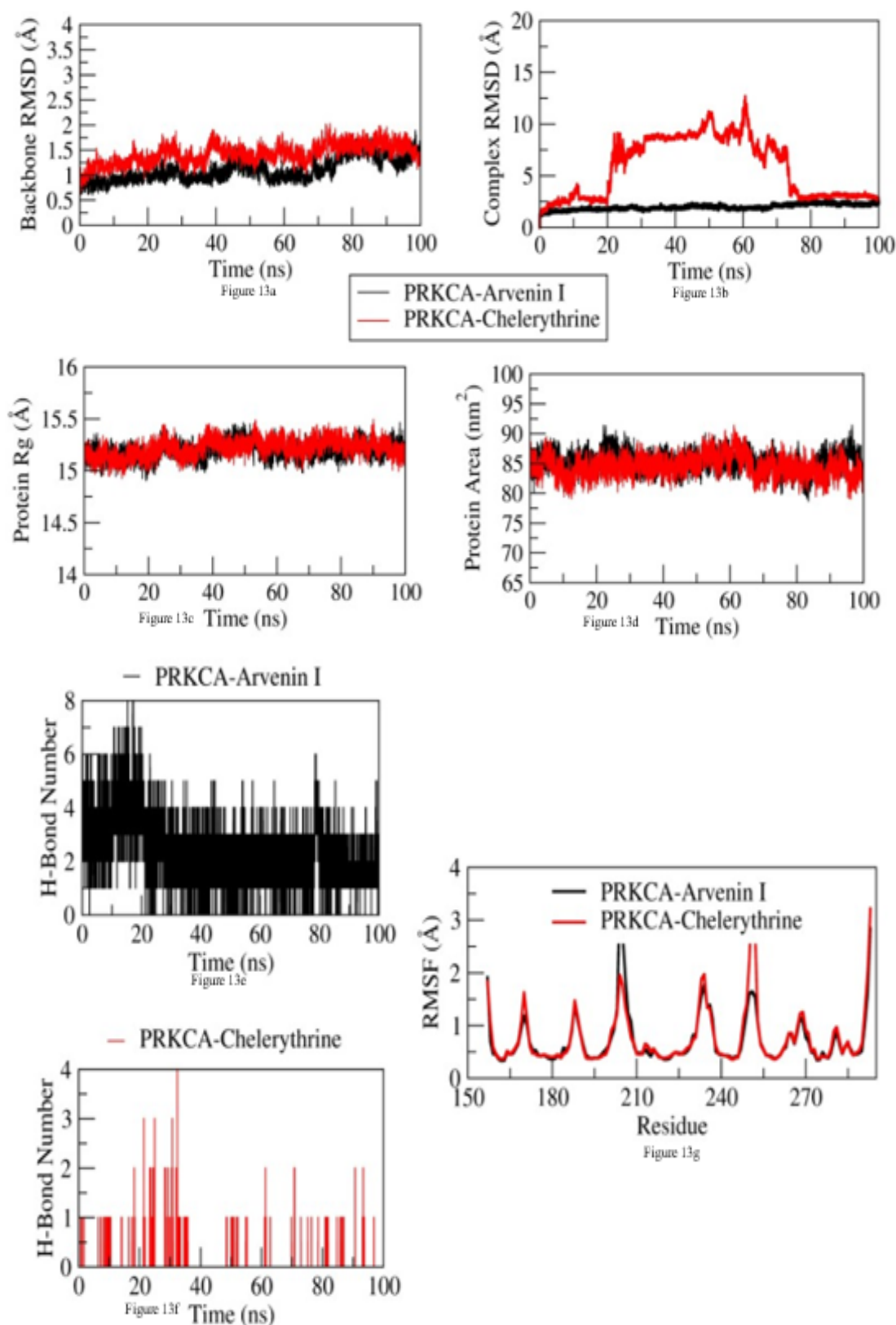


Figure 13: MD simulation for complex PRKCA – Arvenin I and complex PRKCA – Chelerythrine. a) Backbone RMSD b) Complex RMSD c) Rg of the complex d) SASA plot e) Hydrogen bond between PRKCA-Arvenin I f) Hydrogen bond between PRKCA-Chelerythrine g) RMSF of the target protein in the complexes.

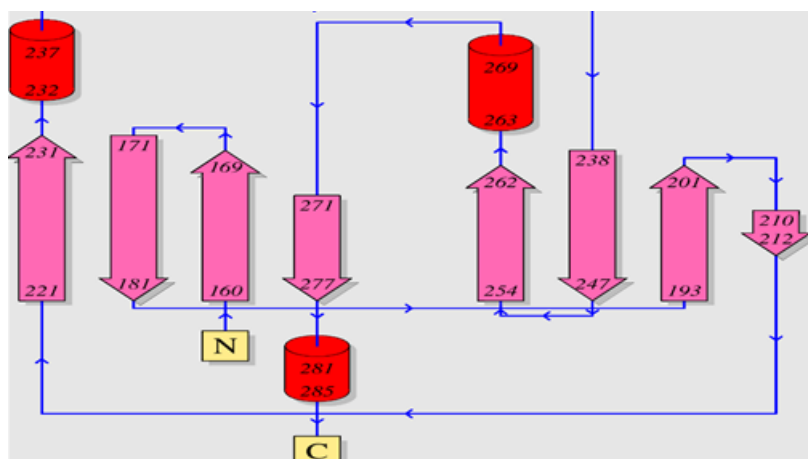


Figure 14: The secondary structure of PRKCA.

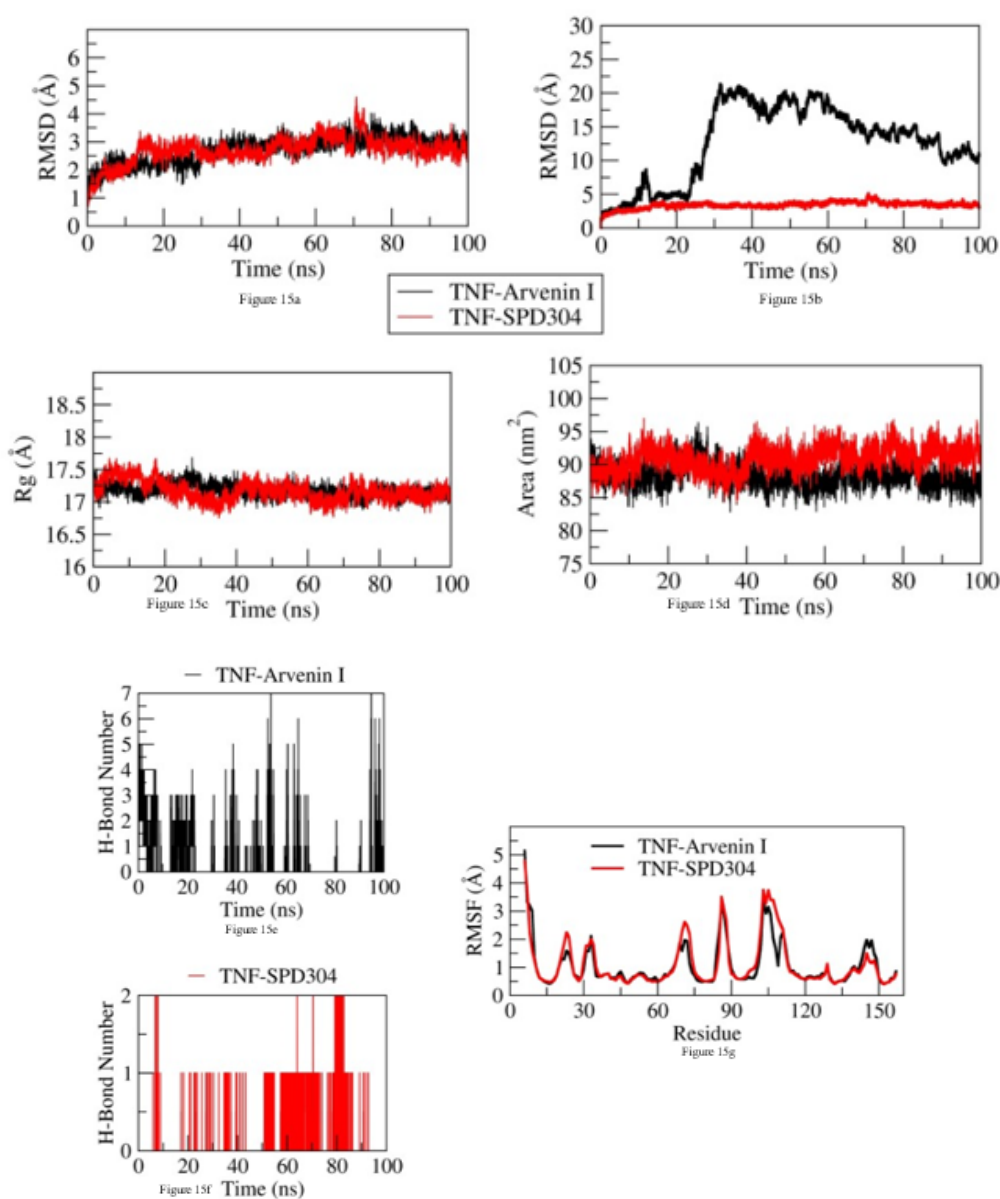


Figure 15: MD simulation for complex TNF – Arvenin I and complex TNF – SPD304. a) Backbone RMSD b) Complex RMSD c) Rg of the complex d) SASA plot e) Hydrogen bond between TNF – Arvenin I f) Hydrogen bond between TNF – SPD304 g) RMSF of the target protein in the complexes.

2.5. Molecular Dynamics simulations:

Based on the molecular docking studies, the docked complexes were subjected to an all-atom Molecular Dynamics (MD) simulation for 100 nanoseconds (ns) in an explicit solvent to examine the docked complex's structural and intermolecular interaction stabilities. Additionally, standard molecule complexes were subjected to an MD simulation for 100 ns. The GROMACS ver. 2021.3 (<https://www.gromacs.org>) package was utilized to run MD simulations using the Amber ff99SB-ildn force field [15]. The topological parameters of the ligands and the whole complex were generated using the AmberTools xleap module (<https://ambermd.org/AmberTools.php>). The partial charges of the ligand were generated using antechamber with a "bcc" charge model. The three-site water (TIP3P) model was used for solvation, keeping the boundary condition of 10 Å in a rectangular box. To neutralize the prepared system, counterions were added as required. The energy minimization step was carried out using the steepest descent method. The systems were equilibrated using the "canonical (NVT) and isobaric (NPT)" ensembles for 1 ns. To keep the volume and temperature (300 K) constant during NVT equilibration, a modified Berendsen thermostat method was used. To keep the pressure constant at 1 bar during NPT equilibration, a Parrinello–Rahman barostat was used. In addition, the particle–mesh Ewald approximation was used with a cut-off value of 1 nm to calculate the long-range electrostatic, Van der Waals, and Coulomb interactions. Similarly, using the LINear Constraint Solver method, bond lengths were constrained. Finally, the system was subjected to a 100 ns MD production run, and the coordinates were recorded every 2 fs. Using the built-in GROMACS tools, the produced trajectories were examined. RMSD was calculated to assess the overall structural stability of the protein–ligand complexes throughout the simulation.

RMSF was evaluated to determine residue-wise flexibility and local fluctuations in the protein structure. The radius of gyration (Rg) was analyzed to examine the compactness and conformational stability of the complexes. SASA was calculated to assess changes in the solvent-exposed surface area during the MD simulation. These parameters were used to investigate the stability and fluctuations of ligand-protein

interactions during the MD simulation (Supplementary Figs. 12–14).

3. RESULTS

3.1. Target identification of retrieved phytoconstituents from *P. kurroa*

A total of 55 phytoconstituents from *P. kurroa* were identified and subjected to target prediction analysis; BindingDB was used to identify the potential protein targets. Among these compounds, Arvenin I and Arvenin II exhibited the highest number of predicted protein targets at a cutoff probability value of ≥ 0.7 , indicating their potential for broad biological activity. The modulation of 108 proteins showed a direct association with T2DM, which probably plays a vital role in the pathogenesis of diseases. Most of the proteins targeted are associated with important cellular processes, including stress response, signal transduction, inflammation, and disease progression. Figure 1 revealed a compound target interaction network of the phytoconstituents. The results are shown in Table 4.

3.2. Network analysis

Using the Cytoscape tool, a network was constructed showing the interactions between the phytoconstituents, proteins, and pathways implicated in T2DM. In the network illustration (Figure 2), the core layer is made of the phytoconstituents, the second layer is of the target proteins, and the third layer is of the disease pathways. From the network represented in Figure 2, the important phytoconstituents identified were Arvenin I and II, and the most important target proteins were Heat Shock Protein 90 AA1 (HSP90AA1), Tumour Necrosis Factor (TNF), and Protein Kinase C (PRKCA). The prominent disease pathways associated with the target proteins are pancreatic secretions, insulin secretion, and vascular smooth muscle contraction.

3.3. Docking analysis

Molecular docking was performed to validate the interactions of the network derived phytoconstituents, Arvenin I and Arvenin II, with the key targets HSP90AA1, PRKCA, and TNF. The docking scores are shown in Table 4 (Figs. 3–11).

Table 4: Comparative molecular docking parameters of Arvenin I, Arvenin II, and reference inhibitors against HSP90AA1, PRKCA, and TNF.

Protein target	PDB ID / UniProt ID	Ligand / reference	PubChem ID	Binding affinity (kcal/mol)	Interacting residues	Figure
HSP90AA1 (HSP90)	1BYQ / P07900	Arvenin I	6441104	−7.3	Asn51, Gly137, Thr184, Gly97	Fig. 3
HSP90AA1 (HSP90)	1BYQ / P07900	Arvenin II	101306925	−7.3	Asn106, Lys58, Gly137, Gly132, Ser113	Fig. 4

HSP90AA1 (HSP90)	1BYQ / P07900	Ganetespi (reference inhibitor)	135564985	-8.3	Tyr139, Gly135, Thr184, Ser52, Met98, Ala55	Fig. 5
PRKCA	4DNL / P17252	Arvenin I	6441104	-7.5	Arg159, Thr250	Fig. 6
PRKCA	4DNL / P17252	Arvenin II	101306925	-7.2	Asp187, Asp248, Thr250, Asp254, Arg252, Phe255	Fig. 7
PRKCA	4DNL / P17252	Chelerythrine (reference inhibitor)	2703	-6.9	Arg249, Asp187, Pro188	Fig. 8
TNF	TNF1 / P01375	Arvenin I	6441104	-7.3	Tyr141, Arg138, Leu142, Pro139, Gly24, Lys65	Fig. 9
TNF	TNF1 / P01375	Arvenin II	101306925	-7.2	Gly24, Ala22, Pro139, Leu142, Lys65, Pro106	Fig. 10
TNF	TNF1 / P01375	SPD304 (reference inhibitor)	126941	-6.4	Arg103, Cys69, Gly68, Trp114, Lys112, Pro113	Fig. 11

Based on the molecular docking results, Arvenin I and Arvenin II showed favourable binding affinities toward HSP90AA1, PRKCA, and TNF, with binding scores ranging from -7.2 to -7.5 kcal/mol. Arvenin I and Arvenin II showed equivalent binding energies of -7.3 kcal/mol, although lower than the reference inhibitor ganetespi (-8.3 kcal/mol). Their interactions with residues such as Gly137 and Thr184 suggest binding within the relevant functional pocket. Arvenin I exhibited binding affinities of -7.3, -7.5, and -7.3 kcal/mol against HSP90AA1, PRKCA, and TNF, respectively, whereas Arvenin II showed corresponding affinities of -7.3, -7.2, and -7.2 kcal/mol. Considering the overall docking profile across the three

network-derived targets, Arvenin I was selected for molecular dynamics simulation to further evaluate the stability and persistence of its interactions under dynamic conditions.

3.4. Molecular Dynamics Simulation

Based on docking data, Arvenin I in complex with HSP90AA1 (HSP90), PRKCA, and TNF was subjected to MD simulation for up to 100 ns. Further, MD simulations are essential for establishing the stability, specificity, and functional inhibitory effects of these interactions. Table 5 represents the results of the simulation.

Table 5: Comparative molecular dynamics analysis of Arvenin I and reference ligand complexes with HSP90AA1, PRKCA, and TNF during 100 ns simulations.

Protein target	Ligand / reference	Backbone RMSD	Complex RMSD	Rg	SASA	H-bonds	RMSF / binding-site residues	Figure
HSP90A A1 (HSP90)	Arvenin I	Fluctuations persisted until ~50 ns, followed by stabilization at ~2.8–3.0 Å, indicating attainment of a stable protein backbone conformation.	Stabilized after ~50 ns at ~3.03–3.5 Å, indicating convergence of the protein–ligand complex after an initial equilibration period.	Maintained ~17.8 Å after initial fluctuations, indicating preservation of the overall compactness of HSP90.	Stabilized within ~112–122 nm ² , indicating comparable solvent exposure throughout the equilibrated trajectory.	Maximum of 7; approximately 3–4 maintained on average, indicating persistent ligand–protein hydrogen-bonding interactions.	Asn51, Gly97, Gly137, and Thr184 showed relatively low fluctuations, supporting stable occupancy of the binding region.	Fig. 12a–g

		on during the latter half of the simulation.						
HSP90A A1 (HSP90)	Ganetespib (reference)	Stabilized earlier, after ~20 ns, at ~2.0–2.5 Å, indicating relatively rapid backbone equilibration.	Converged after ~18 ns and remained at ~2.5–3.0 Å, indicating stable complex dynamics.	Maintained ~17.0 Å, demonstrating preservation of global protein compactness.	Remained within ~112–122 nm ² , indicating stable solvent exposure.	Maximum of 5; approximately 3 on average, demonstrating sustained hydrogen-bond interactions with HSP90.	Ser52, Ala55, Met98, Gly135, Tyr139, and Thr184 showed relatively low fluctuations within the interaction region.	Fig. 12a–g
PRKCA	Arvenin I	Increased gradually from the initial state and stabilized after ~70 ns at ~1.5 Å, indicating a stable PRKCA backbone during the equilibrated phase.	Remained around ~1.5 Å until ~70 ns, followed by stabilization at ~2.5 Å, indicating comparatively smooth complex convergence.	Remained within ~15.1–15.4 Å, demonstrating preservation of PRKCA compactness.	Fluctuated within ~80–90 nm ² , indicating relatively stable solvent exposure.	Maximum of 8; approximately 3–4 on average, indicating persistent intermolecular hydrogen bonding.	Arg159, Asp187, Pro188, Asp248, Arg249, and Thr250 exhibited relatively low fluctuations, consistent with a conformationally stable interaction region.	Fig. 13a–g
PRKCA	Chelerythrine (reference)	Stabilized after ~70 ns at ~1.5 Å, indicating stable backbone dynamics after equilibration.	Increased to nearly ~10 Å after ~20 ns and converged to ~2.5 Å only after ~80 ns, indicating substantial early structural rearrangement and delayed complex equilibration.	Remained within ~15.1–15.4 Å, indicating preservation of global compactness.	Remained within ~80–90 nm ² , showing comparable solvent exposure to the Arvenin I complex.	Maximum of 4; fewer than 1 on average, with hydrogen bonds absent at several time points.	Asp187, Pro188, and Arg249 showed relatively low fluctuations within the interaction region.	Fig. 13a–g
TNF	Arvenin I	Increased gradually and stabilized after ~70 ns at ~3.0 Å, indicating	Increased markedly to nearly ~20 Å after ~22 ns and subsequently	Remained close to ~17.2 Å, indicating that global TNF compactness	Stabilized near ~87 nm ² , indicating relatively stable global	Up to 7 hydrogen bonds were observed at isolated time points, with ~3	Gly24, Lys65, Arg138, Pro139, Tyr141, and Leu142 showed relatively low	Fig. 15a–g

		stable TNF backbone dynamics.	decreased to ~10 Å without a stable plateau, indicating substantial rearrangement and lack of stable complex convergence.	s was largely preserved despite complex instability.	solvent exposure.	during the earlier trajectory; no hydrogen bonds were detected between 70–100 ns, indicating loss of persistent ligand contacts.	fluctuations, although local residue stability did not correspond to stable overall ligand binding.	
TNF	SPD304 (reference)	Stabilized after ~70 ns at ~3.0 Å, indicating stable TNF backbone dynamics.	Remained approximately ~2.5 Å throughout the simulation, indicating a stable and well-converged protein–ligand complex.	Maintained ~17.2 Å, demonstrating preservation of TNF compactness.	Stabilized near ~92 nm ² , indicating stable solvent exposure.	Maximum of 2; fewer than 1 on average, indicating limited but persistent hydrogen-bond formation.	Gly68, Cys69, Arg103, Lys112, Pro113, and Trp114 constituted the reported interaction region, with the complex nevertheless maintaining a stable trajectory.	Fig. 15a–g

The parameters included complex stability, RMSD behavior, maintenance of protein compactness (Rg), solvent accessible surface area (SASA), hydrogen bond interactions, and the stability of residues involved in ligand binding. Arvenin I showed favorable dynamic behavior with HSP90AA1 and PRKCA, with stable post-equilibration complex RMSD profiles, relatively consistent Rg and SASA values, and sustained hydrogen bond interactions. The PRKCA–Arvenin I complex additionally showed a maximum of eight hydrogen bonds with an average of approximately 3–4 interactions, while the interacting residues exhibited relatively low fluctuations. Although the TNF–Arvenin I complex did not maintain a stable complex RMSD plateau and hydrogen bond interactions were lost during the later stage of the simulation, the overall MD profile demonstrated favourable stability with two of the three investigated targets. Therefore, considering the combined stability parameters rather than a single MD metric, Arvenin I was prioritized for further preclinical studies.

4. DISCUSSION

Arvenin I and II, phytoconstituents of *P. kurroa* were identified as the most important phytoconstituents targeting HSP90AA1, TNF, and PRKCA, which are metabolically related to type II diabetes. A systematic network pharmacology workflow was used to identify potentially active phytoconstituents relevant to type 2 diabetes mellitus. From 55 phytoconstituents compiled from the

literature and curated databases, 31 compounds met the Molsoft drug-likeness cutoff of ≥ 0.06 . Their predicted toxicity profiles were evaluated to support early-stage assessment of safety and drug development potential. High-confidence putative protein targets were identified using BindingDB at a prediction score cutoff of ≥ 0.7 . STRING and KEGG analyses linked these targets to type 2 diabetes associated pathways, supporting the multi-component and multi-target therapeutic potential of *P. kurroa*.

Among the predicted targets, Arvenin I is of special interest because PRKCA encodes protein kinase C α , and has been linked to impaired insulin signaling. Glycated albumin impaired insulin-induced IRS signaling and glucose metabolism through a protein kinase C α -mediated mechanism in skeletal muscle cells [8]. Protein kinase C activation has also been associated with vascular complications of diabetes [9]. TNF- α is also relevant to T2DM; elevated serum TNF- α has been associated with glycemic control and insulin resistance in people with T2DM [20]. Akash MSH et al (2018) have also described its role in insulin resistance and T2DM pathogenesis [25]. However, there is no direct supporting evidence with respect to the specific role of HSP90AA1 in diabetic inflammation or complications. Overall, these findings make Arvenin I a candidate for further investigation. Among these, Arvenin I appears particularly relevant because PRKCA is implicated in insulin signaling, pancreatic secretion, metabolic stress, and insulin

resistance, while TNF and HSP90AA1 are also linked to diabetic inflammation and complications [10,11,17,18,19,21,22,23,24].

The present findings extend earlier work on *P. kurroa* by moving from extract-level antidiabetic evidence to a constituent-target framework. Previous studies have shown that *P. kurroa* extracts improve glucose homeostasis, oxidative stress, insulin sensitivity, and GLUT4-related signaling in experimental diabetes models [25, 26, 27, 28]. More recent work has also supported the role of *P. kurroa* in diabetic nephropathy through network pharmacology, molecular docking, and in vitro validation [27]. In addition, the phytochemical and pharmacological literature has consistently recognized *P. kurroa* as a source of bioactive iridoid glycosides with translational potential [29]. In this context, our findings contribute an additional mechanistic perspective by recognizing Arvenin I and II as potential ligands for HSP90AA1, PRKCA, and TNF. Arvenin I was selected as the final candidate based on docking and MD simulations. This demonstrated stable dynamic behavior of the HSP90AA1– and PRKCA–Arvenin I complexes, with preserved compactness and sustained hydrogen-bond interactions.

Although the TNF–Arvenin I complex showed favourable docking, it lacked stable complex-RMSD behavior and lost hydrogen-bond interactions during the later simulation phase. Thus, the integrated docking and MD results, particularly the favourable stability with HSP90AA1 and PRKCA, provided the computational basis to suggest Arvenin I for further studies leading to clinical applications. The results from docking and molecular dynamics indicate that Arvenin I, specifically, might be the more advantageous molecule for targeting PRKCA and TNF [30].

Importantly, the current results strengthen the biological plausibility of Arvenin I as a candidate multi-target therapeutic antidiabetic molecule. Before any translational step, Arvenin I should undergo in vitro target validation, *in vivo* efficacy testing, toxicity profiling, and pharmacokinetic assessment.

5. CONCLUSION

The present study investigated the effect of individual phytoconstituents from the medicinal plant *P. kurroa* for putative anti-diabetic activity. Of the total 55 phytoconstituents, two were shortlisted: namely Arvenin I and II, and the protein targets that interact with them: HSP90AA1, PRKCA, and TNF. These proteins have been implicated in diabetes-associated metabolic pathways involved in pancreatic secretion, insulin secretion, and vascular smooth muscle contraction. It is observed that Arvenin I interacts effectively with the proteins HSP90AA1 and PRKCA, as verified by molecular dynamics analysis. It demonstrates the binding capabilities of the phytoconstituent to the protein targets and its stability. Hence, it is hypothesized that we can use this as a lead molecule for the treatment of these metabolic disorders. Further, preclinical studies need to be conducted to verify the *insilico* results for therapeutic purposes.

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Declaration of competing interest

The corresponding author confirms on behalf of all authors that there are no known conflicts of interest associated with the publication.

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