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RESEARCH ARTICLE OPEN ACCESS

Antioxidant capacity and drug-like activity of *Artocarpus heterophyllus* against D3, DJR-1, and SOD-1: A Computational exploration

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ABSTRACT
The current lifestyle and rising health concerns have increased the demand for nutraceuticals (nutrition + pharmaceuticals) food and food products, providing health benefits beyond essential nutrition. Advances in research and technology has enhanced the quality of life, allowed us to fight diseases, and enhanced longevity. Unfortunately, due to the heightened longevity it leads to rise in the incidences of age-related diseases such as Parkinson Disease (PD) arising through cellular, genetic, and multifactor pathophysiological processes. Treating PD using antioxidants by modulating oxidative stress via interaction with DJR 1 protein and D3R could be a best strategy. In this study 32 polyphenolic compounds from Jackfruit (*Artocarpus heterophyllus*) were evaluated using prediction of molecular properties and in silico docking studies. All selected compounds were subjected to ADMET followed by docking to investigate newly designed proteins' binding affinities and conformations. Extensible toxicity prediction protocol of Discovery studio (2019) used to predict their molecular properties, important to be a drug candidate. Molecular docking simulations of Jackfruit with novel designed PD and ROS associated protein has been the focus of our work. Artonin K and Artonin S were the most potent inhibitors as for pharmacokinetic, pharmacodynamics, physicochemical, and docking properties.

Keywords: *Artocarpus heterophyllus*. ADMET. Binding affinity. In silico analysis. Molecular Docking simulation. Parkinson's disease.

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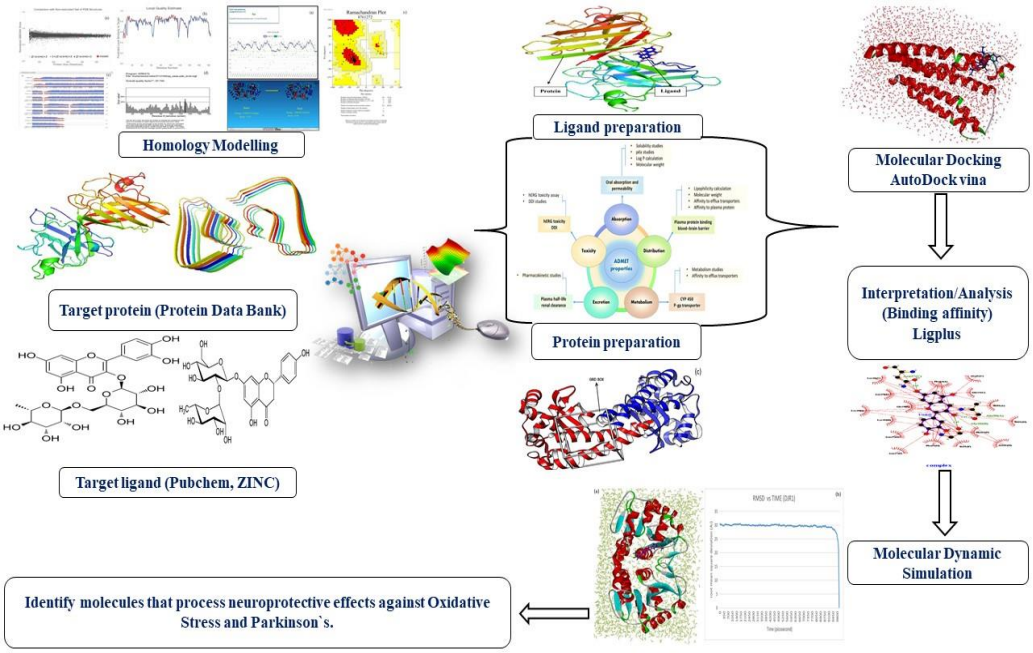
GRAPHICAL ABSTRACT



I. INTRODUCTION

Formation of Lewy bodies and a decrease in the number of neurons characterized Parkinson's disease (PD) as the second most prevalent neurodegenerative disorder, followed by Alzheimer's disease which impacts over 10 million patients worldwide (Poewe *et al.,* 2017). PD results from the progressive dopamine neurons depletion selectively from the substantia nigra region of the ventral midbrain. The cellular symbols of PD are intracellular proteinaceous insoluble inclusions in the presynaptic termini named Lewy bodies, formed by α-syn aggregates (Osterhaus *et al.,* 1997). Parkinson's disease is a neurological movement disorder marked by impaired balance, bradykinesia, rigidity, and the presence of resting tremors. In addition to deficits in movement, PD patients can also exhibit non-motor symptoms including depression, apathy, anxiety, dementia, constipation, disrupted sleep etc. (Vlachos & Tavernarakis, 2010)

PD and oxidative stress have been studied to have significant association. Oxidative stress means a loss of equilibrium between the levels of reactive oxygen species (ROS) produced and the number of reactive intermediates detoxified by the biological system. Oxidative stress can lead to enhanced reactive oxygen species (ROS) generation, and its accumulation increases oxidized proteins, lipids, and DNA levels. Its cumulative effect results in cellular degeneration and impaired regeneration which is fundamentally associated with aging-related degenerative diseases (Dias *et al.,* 2013). ROS and RNS (Reactive nitrogen species) are generated and accumulate over time during cellular stresses or normal metabolic processes. Under normal conditions, cells are well equipped with anti-oxidant defense mechanisms to resist oxidative stresses thereby maintaining safe levels of free radicals. Whereas in the brain tissue of PD patients, higher levels of ROS and other free radicals (e.g., superoxide anions, nitric oxide, hydrogen peroxide, singlet oxygen, peroxyl, and, hydroxyl) are present indicating their importance (Puspita *et al.*, 2017).

The regulation of ROS has become a major research target for the prevention and treatment of neurodegenerative diseases, especially PD. Antioxidant can neutralize pro-oxidant molecules or free radicals or their actions, thus attenuating oxidative stress. Flavonoids and polyphenols are major antioxidant molecules in fruits and vegetables (Sheoran *et al.,* 2013). These flavonoids and polyphenols are potently scavenged ROS and RNS (Reactive nitrogen species) and play important roles in aging-related degenerative diseases (Ivanova *et al.,* 2018). This positive characteristic can be attributed to phytochemicals such as carotenoids, alkaloids, vitamins, minerals, and polyphenols. As phytochemicals have a wide range of chemical, biochemical, and molecular characteristics, they are of considerable interest for treating neurodegenerative diseases. These compounds directly contribute to the antioxidant capacity and are usually used in the prevention of oxidative rancidity (Gupta *et al.,* 2016).

Some of the targets for potential

therapeutics in PD are Parkin, DJ1, PINK1, LRRK2, SNCA Motif, and dopamine receptors. DJ1 is a neuroprotective protein that regulates anti-oxidant, anti-inflammatory, and anti-apoptotic pathways and protects cells from oxidative stress. It acts as a ROS quencher and molecular chaperone. SOD-1 (superoxide dismutase) is an antioxidant defense enzyme involved in the detoxification of superoxide radicals to reduce oxidative stress. It converts O_2•– to hydrogen peroxide (H_2O_2), which is then converted to H_2O and O_2 (Sehgal et al., 2018). The importance of Dopamine D3R in the early development and occurrence of PD cannot be neglected. Agonistic stimulation of D3R Increases dopamine concentration, decreases α-Syn accumulation, enhances secretion of brain-derived neurotrophic factors (BDNF), ameliorates neuroinflammation, mitigates oxidative stress, and promotes neurogenesis in the nigrostriatal pathway. Research and studies have shown evidence that activation of the D3 receptor is beneficial for the treatment of PD. Dopamine receptor agonists act by stimulation of presynaptic and postsynaptic DA receptors (Hisahara & Shimohama, 2011).

Jackfruit (Artocarpus heterophyllus) is a tropical tree, local to India and usually placed in Asia, Africa, and numerous areas in South America. The fruit, bark, leaves, and roots are endowed with healing attributes and are applied inside the many conventional medicinal structures for the control of diverse disarrays. Based on preclinical studies, jackfruit is famous, for anti-melanin, antimicrobial, antidiabetic, antioxidant, immunomodulatory, antiviral, anthelmintic, wound-healing, anti-inflammatory, and antineoplastic activities. Due to the presence of many active ingredients, it possesses strong antioxidant activity; has phenolic compounds and flavonoids like oxyresveratrol, deconic acid, morin, hesperidin, apegenin, myristic acid, betulinic acid, laurin acid, alpha-tocophenol, mallic acid, oleic acid, artocarpesin, cyanomaclurin, artocarpanone, norartocarpetin, mulberrin, heteroartonin-A, heteroflavanone, artonin etc. In our work, we have used Jackfruit (Artocarpus heterophyllus), as it can possess strong antioxidant activity against DJ1 protein, superoxide dismutase (SOD-1) enzyme, and dopamine D3 receptors, by using a computational approach.

II. MATERIALS AND METHODS

Ligand and Protein Structure Retrieval

All the flavonoids and polyphenols of *Artocarpus heterophyllus* were identified from an online database- PCIDB (PhytoChemical Interactions DataBase). In the present study, 36 ligands (phytochemicals) were selected and the 3D structure of all the 36 ligands were obtained from the PubChem and ZINC database. The downloaded ligand files were converted from SDF format to PDB format with the help of the Open Babel tool. Visualization of molecular structure of compounds was done using Pymol viewer. The 3D structure of SOD1 enzyme of *C. elegans* (PDBID: 3DC6) was obtained from Protein Databank and was visualized in PyMol software. Due to lack of availability of 3D assembly of DJR-1 and dopamine D3 in the Protein databank database, the 3D structure was built via homology modelling. All the flavonoids and polyphenols of *Artocarpus heterophyllus* were identified from an online database- PCIDB (PhytoChemical Interactions DataBase). In the present study, 36 ligands (phytochemicals) were selected and the 3D construction of all the 36 ligands were obtained from the PubChem and ZINC database. The downloaded ligand files were converted from SDF format to PDB format with the help of the Open Babel tool. Visualization of molecular structure of compounds was done using Pymol viewer.

Homology Modelling

Three-dimensional structure of DJR-1 and Dopamine receptor D3 is not yet available, so to build the homology model of DJR-1 protein, the amino sequence of Glutathione-independent glyoxalase DJR-1.1 of *Caenorhabditis elegans* (GeneBank_ID: NP_493696.1) with 187 amino acid was selected as the query sequence from the NCBI database. The NCBI BLASTp search of query sequence was performed to find the template for modeling. The homology model was built using the SWISS-MODEL program on the bases of template 3SF8.1A (Protein DJ-1, *Homo sapiens*). The amino sequence of Dopamine receptor D3 of *Caenorhabditis elegans* (GeneBank_ID: CCD83407.1) with 245 amino acids was retrieved from the NCBI database and was utilized as a query sequence for template search using the NCBI BLASTp tool. The homology model was built using the template 6CM4.1A (chain A D2 dopamine receptor, *Homo sapiens*) with the help of SWISS MODEL program (Pagadala *et al.*, 2017).

After the model was built on the basis of this template by SWISS-MODEL, structural analysis of the modeled protein structure was done to check the superiority of the model and for validation of various stereochemical aspects. It was done using PROCHECK, ERRAT, and VERIFY 3D on SAVES online server. Finally, the modeled protein structure was imperiled to dynamism minimization by YASARA software.

Analysis of Molecular Descriptor

Molecular properties, including molecular weight, hydrogen bond count (donor and acceptor), octanol-water partition coefficient (log P), number of rotatable bonds, number of rings, number of aromatic rings, and molecular polar surface area, were investigated for all newly designed compounds using the Discovery Studio 3.1 client package developed by Accelrys, based in San Diego, USA (muniba faiza, 2019).

ADMET Screening and Toxicity Studies

ADMET properties study was done to retrieve the information about drug likeness of ligands as it provides an insight into the physiochemical parameters like solubility, lipophilicity, permeability, hydrogen bonding, bioavailability, gastrointestinal absorption, and ability to cross the blood-brain barrier. On the basis of Lipinski's rule of five [molecular mass < 500 Dalton, H-bond donors < 5 (OH and H), H-bond acceptors < 10 (N and O) and Log P (octanol-water partition coefficient) < 5], Veber rule [rotatable bonds < 10] and Ghose rule [Log P between -0.4 and 5.6, molecular weight between 160 and 480, molar refractivity between 40 and 130] the ligands were screened. The ADMET properties were obtained from the SWISS-ADME, pkCSM program (Lipinski *et al.*, 1997).

Optimization of Protein and ligand Structure and Binding Site Prediction

Protein structure optimization was done using UCSF Chimera software, it involves the removal of hetero-atoms, and the addition of polar hydrogen and gasteiger charges. Ligand structure optimization involves the addition of water molecules and charges.

The active binding site on the protein structure was predicted using the F-pocket program. The protein pdb file was uploaded in the server and it showed the various binding pockets present in the protein structure. The superlative binding pocket was selected and the amino acid residues involved were retrieved (Muniba, 2016).

Analysis of Molecular Docking

The optimized or prepared protein and ligand files were employed for molecular docking, aiming to examine the interactions between the protein and ligand molecules. The protein-ligand docking was achieved via the UCSF Chimera and AutoDock Vina v.1.2.0. software. The docking site on the target protein identified using F pocket was expressed by forming a grid box around the region. The ligand was directed onto this protein binding site. Every ligands were docked with the 3 proteins one by one and were analyzed to determine the binding conformation and energy value. The resultant protein-ligand complex was observed in PyMol software (Padariya *et al.,* 2014).

After docking, the ligand-protein interaction was studied using ligplot+ software which generates a schematic illustration of the ligand-protein interaction. It locates the specific contact between the atoms of the ligand and amino acids of the protein and analyzes hydrophobic interactions and hydrogen bonding between protein and ligand. In AutoDock, the free energy binding is determined by derivation of the pragmatic requisite free energy function. AutoDock uses an expanded "master equation" to archetypal the unrestricted dynamism of requisite, count entropic terms to the molecular mechanics equations (Morris *et al.,* 1998).

$\Delta G = \Delta Gvdw + \Delta GH\neg bond + \Delta Gelec + \Delta Gconform + \Delta Gtor + \Delta Gsol$

The initial four terms represent the standard molecular mechanics parameters for dispersion/repulsion, hydrogen bonding, electrostatics, and deviations from covalent geometry, respectively. ΔGtor models the constraints on internal rotors and accounts for global rotation and translation, and ΔGsol simulates the desolvation effect during binding, including the hydrophobic effect, which involves solvent entropy changes at solute-solvent interfaces (Morris *et al.,* 1998). The protein structure (PDB: 2Y9X, Chain: A) was converted into. pdbqt file by adding atom types using MGLTools and ligand pdb files were transformed into the corresponding. pdbqt format by the identical software. The polar hydrogens and Kollman united atom charges were added to the protein structure using AutoDock tools. All rotatable bonds in the ligands were kept free to allow flexible docking. AutoGrid was working to build grid maps around the protein binding sites in which, grid size was set to 74×74×74 grid points (in X, Y, Z) and spacing between grid points was kept 1. Lamarckian Á genetic algorithm was preferred to search for 250 best ligand conformers. Standard docking protocol \ was applied with a population size of 75 million maximum energy evaluations. For each docking simulation, 200 independent docking runs were carried out for each ligand generated by using genetic algorithm searches. All the other parameters were set to their default values (Babel, 2019).

Molecular Dynamics Simulation

The protein-ligand complex exhibiting the highest binding affinity was selected based on the docking study results. Subsequently, it underwent molecular dynamics simulation to investigate the

stability of the protein-ligand interaction. It was carried out using Biovia Discovery Client V19.1.0.18287 software.

For MD simulation, CHAMM36 forcefield was used and the steepest decent algorithm was applied to energy minimization for 3000 cycle steps. Equilibration was performed at a simulation time of 400 ps, to relax the solvent in protein structure under constant temperature dynamic (NVT) conditions. The production simulation was done at a simulation time of 10000 ps under a constant temperature dynamic (NVT). The temperature of all simulations was set to 300K and all MD frames were saved at every 50 ps for trajectory analysis. Trajectory analysis of MD conformations was calculated for root mean square deviation (RMSD) and root mean square fluctuation (RMSF) (Habibyar *et al.*, 2016).

III. RESULTS AND DISCUSSION

To reveal the role of SOD-1 enzyme, dopamine D3 receptors and DJR1 protein in further unexplored and new fields, much more research on these proteins is needed which can be useful for designing enzymatic activities for various applications (Nadeem Khan, 2019). The active ingredient of *Artocarpus heterophyllus* could be illustrated as a natural source for the most active PD and ROS inhibitors. Several studies have determined that the number and position of the hydroxyl group plays a key role in the inhibition efficacy. The inhibitor Hydroxyl group carries out the nucleophilic attack on the copper atoms, which are localized in the enzyme on its active site and are unswervingly intricate in the transferring protons during catalysis, which later results in minimizing ROS (Gutierrez-Zepeda *et al.*, 2005). Thirty-Six, novel inhibitors are used considering all this knowledge to improve their efficiency.

Homology Modeling of 3D structure of DJR1

The homology model of DJR-1 was built using the template 3SF8.1A (Protein DJ-1, *Homo sapiens*) which was 191 amino acids long. In the SWISS-MODEL template search, the template showed the highest sequence identity of 52.43 and the highest GMQE (Global Model Quality Estimation) and QSQE (Quaternary Structure Quality Estimation) scores of 0.86 and 0.88 respectively. Also, our model had a remarkable Z-score of -0.93. Structural validation of the modeled protein structure to check the model quality and validation of various stereochemical aspects was done using PROCHECK, ERRAT, and VERIFY 3D on the SAVES online server. The Ramachandran analysis revealed that 92% of the amino acid residues plotted in the PROCHECK model were within the most favorable region, with no residues found in the disallowed region. The ERRAT program analyzed the relative frequencies of non-covalent interaction between atoms of various types to approve the protein structure. The overall quality factor of our modeled protein was 97.74, thus it's considered a good high-quality structure. The VERIFY3D program analyzed the coordinates of the atomic structure, which was used to score the compatibility of the 3D structure with the amino acid sequence. The scrutiny of the 3D-1D score of our modeled protein by VERIFY3D showed that 94.05% of the residue had an average of 3D-1D score >= 0.2, thus our model was acceptable. On the basis of these result the archetypal was classified as stable protein structure (FIGURE 1).

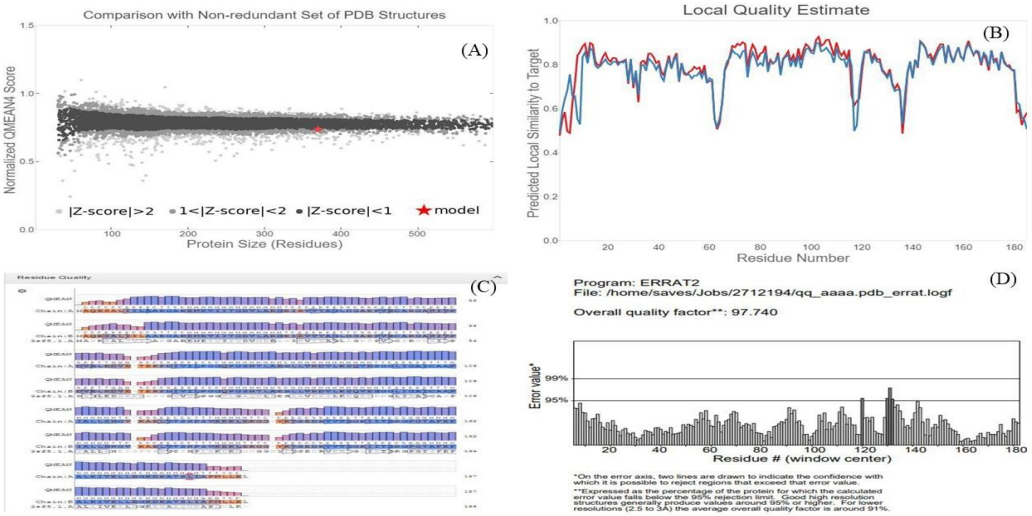


FIGURE 1 - (A) z score of modelled djr-1 protein of modelled djr-1 protein (B) quality estimate (C) structure alignment generated in Swiss- model (D) structural validation of modeled djr-1 protein.

Finally, the modeled protein structure was subjected to minimization of energy, which is required to find a point in configuration space where all the forces on the atom are balanced and a stable configuration is achieved. Yasara software minimized the energy of the protein structure from -135675.3 kJ/mol to -190340.2 kJ/mol (FIGURE 2). This modeled DJR-1 protein 3D structure was further used for docking with ligands.

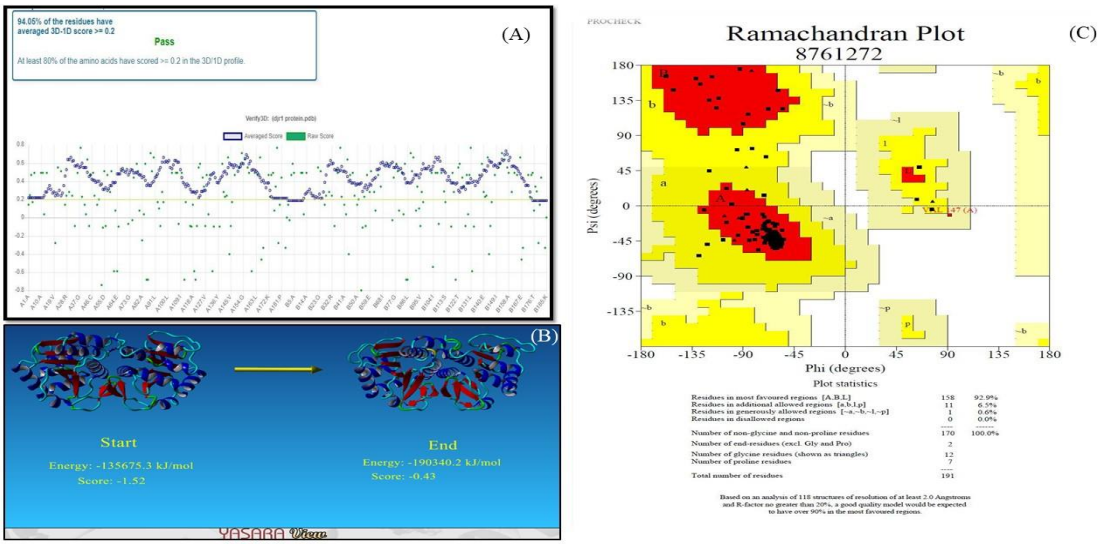


FIGURE 2 - (A) verify3d program result for djr-1 protein (B) energy minimization of djr-1 protein structure using yasara (C) Ramachandran plot for modeled djr-1 protein obtained from procheck program, showing the amino acid residues in allowed and disallowed regions.

Modelling of dopamine D3

Homology model of Dopamine D3 receptor was built using the template 6CM4.1A (chain A D2 dopamine receptor, *Homo sapiens*). In the SWISS-MODEL template search, this template showed highest GMQE score of 0.57 and QMEAN (Z score) of -3.59 (FIGURE3).

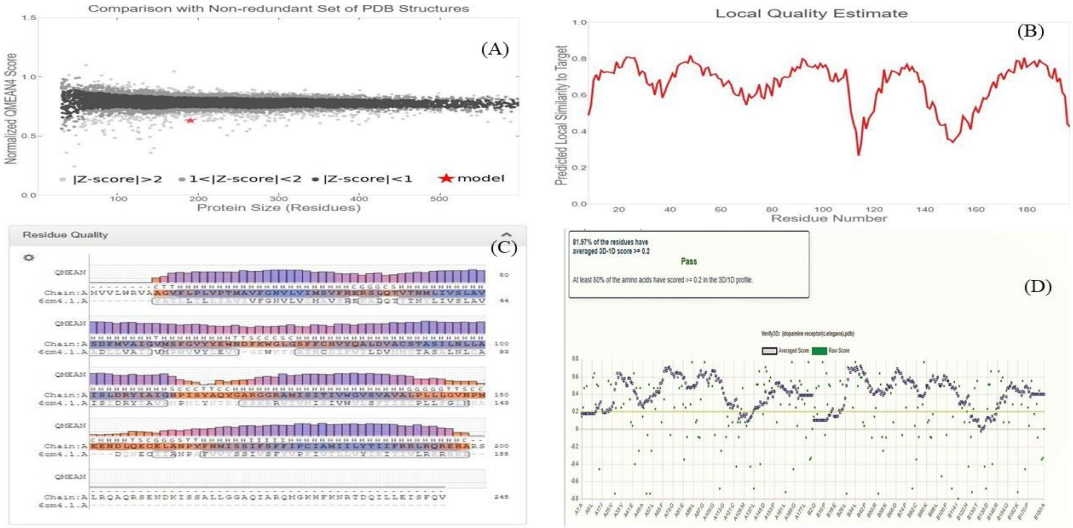


FIGURE 3 - (A) z score of modeled dopamine d3 protein (B) quality estimate of modeled dopamine d3 protein (C) structure alignment generated in swiss- model between template "3sf8.1a" and target sequence of dopamine -d3 protein (D) verify3d program result for dopamine d3 protein.

After the model was built based on this template, the Ramachandran plot obtained from the PROCHECK program showed that 91% of the residues of amino acid of the model were in the maximum auspicious region and only 0.6% of residue was in the disallowed region (FIGURE 4). The ERRAT program result exposed that the inclusive quality aspect of our modeled protein was 95.62, thus it's considered a good-

high quality structure. The VERIFY3D program analysis showed that 81.97% of the residue had an average of 3D-1D score >= 0.2, thus our model was acceptable. On the basis of these results, the model was classified as stable protein structure. Finally, the model was subjected to minimization energy using Yasara software and the energy was minimized from -67650.7 kJ/mol to -99693.7 kJ/mol. This modeled Dopamine D3 receptor 3D structure was further used for docking with ligands.

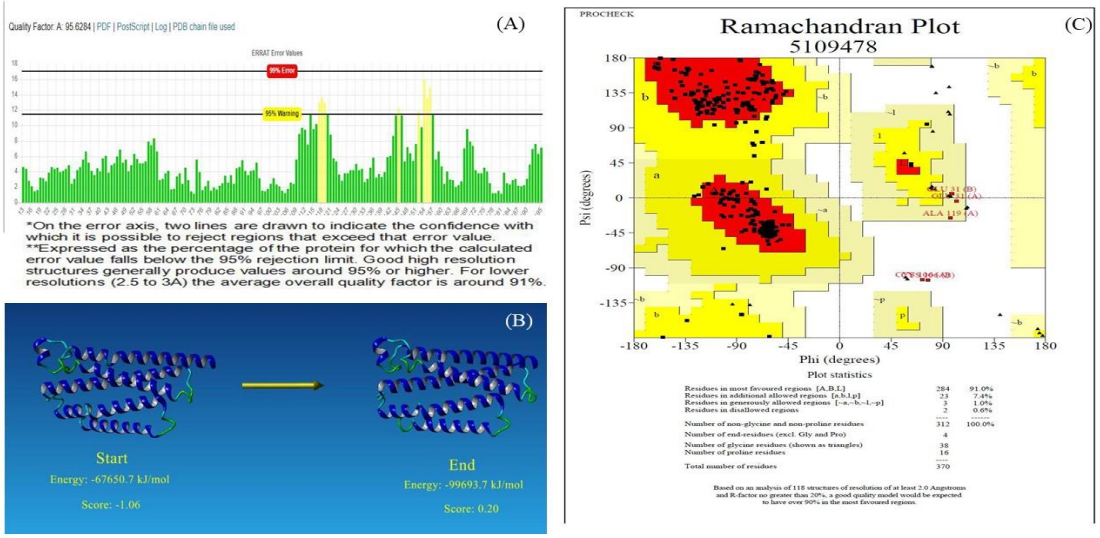


FIGURE 4 - (A) Structural validation of modeled dopamine d3 protein using errat program (B) energy minimization of dopamine d3 protein (C) Ramachandran plot for modeled dopamine d3 protein obtained from procheck program, showing the amino acid residues in allowed and disallowed regions.

Molecular Properties of Bioactive Molecules (Ligands) of *A. Heterophylus*

Molecular physicochemical and drug likeness are the two attributes which provide base for the compound to be an efficient drug candidate (Lipinski *et al.,* 1997). Rules defined by Lipinski and his coworkers is considered as the thumb rule, which describes molecular properties important for a drug's pharmacokinetics in the human body, involving ADME (Tambunan & Wulandari, 2010). Log *P* (an octanol water partition coefficient) is applied as a significant tool in quantitative structure-activity relationship (QSAR) studies and also in rational drug design as a measure of molecular hydrophobicity, Log *P* value less than 5 is preferable for drug likeness property. The favorable range of molecular weight is between 160480 g/mol for drug-likeness properties as stated by Tambunan and Wulandari (Tambunan & Wulandari, 2010). Concerning the number of hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms) and hydrogen bond acceptors (nitrogen or oxygen atoms), values should be 10 or less than 10 and 5 or less than 5, which agrees with the rule number three and four respectively. According to rule number five (Lipinski rules), (Lipinski *et al.,* 1997) the preferred number of rotatable bonds is 15 or less than 15. Bioactive molecules, of *the A heterophylus* this study has well qualified in three rules (Alogp, Molecular weight, and count of rotatable bonds) of Lipinski's filter.

ADMET and Toxicity Profiles

Our work has determined various pharmacokinetic and pharmacodynamic properties of *A. Heterophylus* and its bioactive molecules. Pharmacokinetics properties are aqueous solubility and drug-likeness, blood-brain barrier penetration (BBB), human intestinal absorption, etc (TABLE S1). Pharmacodynamic properties, also known as toxicity profile, encompass carcinogenicity, AMES mutagenicity, aerobic biodegradability, ocular, skin irritancy, and skin sensitization. To analyze the ADME results of newly designed compounds, reference values of the ADMET descriptors are obtained from the SWISS-ADMET & pkCSM ADMET (Daina *et al.,* 2017; Mahnashi *et al.,* 2022).

In the ADME study, when results are compared to the reference level values, all ligands have good or optimal aqueous solubility and better drug-like properties without blood-brain barrier (BBB) penetration. Among 36 compounds, most of the compounds have shown low or moderate intestinal absorption from the *in-silico* toxicity studies, as listed in(TABLE S2), none of the ligands have shown mutagenicity and carcinogenicity. ADMET screening was done based on the five rules of Lipinski's, Veber's rule, and Ghose's rule (Benet *et al.,* 2016). These rules analyze the bioavailability and oral

absorption of the drug and the ligands which do not follow these rules cannot be considered as good potential threptic compounds. According to these three rules, 10 ligands - Artocarpin, Betulinic acid, Cycloartenol, Cyclohetrophyllin, Artonin A, Artonin B, Artonin C, Artonin D, Artonin I, and Artonin X were eliminated and only the remaining 26 ligands were considered for docking with the protein structure.

Molecular Docking and Analysis

Starting from molecular docking of 26 liagnds -Dihyromorin, Morin, Artocarpesin, Cyanomaclurin, Oxidihydroartocarpesin, Artocarpetin, Artocarpanone, Norartocarpetin, Norartocarpin (mulberrin), Isoartocarpin, Cycloartocarpin-A, Oxyresveratrol, Heterophylol, Heteroartonin-A, Kuwanon-T, Artocarpetin-B, Cycloartocarpesin, Heteroflavanone-A, Heteroflavanone-B, Heteroflavanone-C, Artonin -J, Artonin-K, Artonin -L, Artonin-S , Artonin -T and Artonin-U, were selected after screening of ADMET properties. Molecular docking studies were achieved with the parameter of Auto Dock. For docking simulation of tyrosinase, 26 optimized ligands were docked with the enzyme SOD-1 (PDB: 3DC6), DJR-1 and D3. Top-ranked conformations were selected for further analysis and visual interaction studies. Drug likeliness, log *P*, toxicity risks, and molecular weight are used to appraise the compound's inclusive probable to be suitable a ligand as a potential therapeutic entrant. Artonin K emerged as the most active compound, ranking second based on descriptor analysis of ADME and toxicity profiles obtained from the extended protocol of Discovery Studio (TABLE I).

TABLE I - Enzyme- inhibitor binding interactions of 26 novel ligands with the DJR1, Dopamine D3 and SOD obtained in AutoDock Vina.

		DOCKING WITH DJR1 PROTEIN			DOCKING WITH DOPAMINE D3 RECEPTOR			DOCKING WITH SOD1 ENZYME		
Sr. NO.	LIGAND	BINDING ENERGY	No. of H-bonds formed	No. of hydrophobic interaction	BINDING ENERGY	No. of H bonds formed	No. of hydrophobic interaction	BINDING ENERGY	No. of H-bonds formed	No. of hydrophobic interation
1	Dihyromorin	-6.2	5	5	-6.1	2	6	-6.3	5	7
2	Morin	-6.1	3	6	-6.6	2	6	-6.6	4	6
3	Artocarpesin	-6.2	3	6	-6.7	1	12	-7.2	3	8
4	Cyanomaclurin	-6.2	5	4	-6.4	2	5	-6.2	4	3
5	Oxidihydroartocarpesin	-6.2	3	6	-6.8	3	8	-7.1	3	7
6	Artocarpetin	-6.3	1	8	-6.4	2	7	-6.6	2	5
7	Artocarpanone	-6.2	1	8	-6.3	3	6	-6.7	4	7
8	Norartocarpetin	-6.4	4	6	-6.2	1	9	-6.6	2	7
9	Norartocarpin (mulberrin)	-6.6	2	10	-6.9	2	9	-6.8	6	6
10	Isoartocarpin	-7.1	3	11	-6.6	0	7	-7.7	1	13
11	Cycloartocarpin A	-6.7	1	7	-6.4	1	9	-7.8	1	11
12	Oxyresveratrol	-6.0	3	7	-5.8	4	5	-5.8	3	5
13	Heterophylol	-6.4	3	8	-6.7	1	6	-6.7	1	9
14	Heteroartonin A	-6.3	1	8	-6.3	2	8	-7.2	1	11
15	Kuwanon T	-6.1	0	12	-6.2	2	7	-7.3	2	10
16	Artocarpetin B	-6.0	1	11	-6.8	1	8	-6.6	1	9
17	Cycloartocarpesin	-6.7	2	7	-7.4	0	11	-7.5	2	8

18	Heteroflavanone A	-5.7	2	7	-6	2	9	-6.2	1	9
19	Heteroflavanone B	-6.2	2	9	-6	1	9	-6.5	1	9
20	Heteroflavanone C	-6.0	0	12	-6.4	3	7	-6.6	1	7
21	Artonin J	-7.2	1	10	-7.1	2	9	-7.8	3	8
22	Artonin K	-7.4	2	10	-7.5	3	14	-7.9	5	6
23	Artonin L	-7.0	1	7	-7.2	2	6	-7.8	5	8
24	Artonin S	-7.2	4	11	-7.9	3	9	-8.7	7	8
25	Artonin T (Lucuminoside)	-7.1	3	8	-7.0	1	9	-7.6	2	9
26	Artonin U	-6.1	2	7	-7.0	1	8	-6.7	1	8

The analysis of the docking results with DJR1 protein of *C. elega*ns showed that, the ligands Artonin K had the highest binding energy (lowest delta G value) of -7.4 kcal/mol and Artonin-S and Artonin-J had the second highest binding energy of -7.2 kcal/mol. LigPlot+ analysis showed that, Artonin K had formed 2 H-bonds with the residues Ser127, Arg29 and 10 Hydrophobic interactions with the residues Leu179, Ser178, Arg49, Ala48, Pro77, Gln76, Glu16, Gly75, His125 and Pro182 of DJR-1 protein (FIGURE 5).

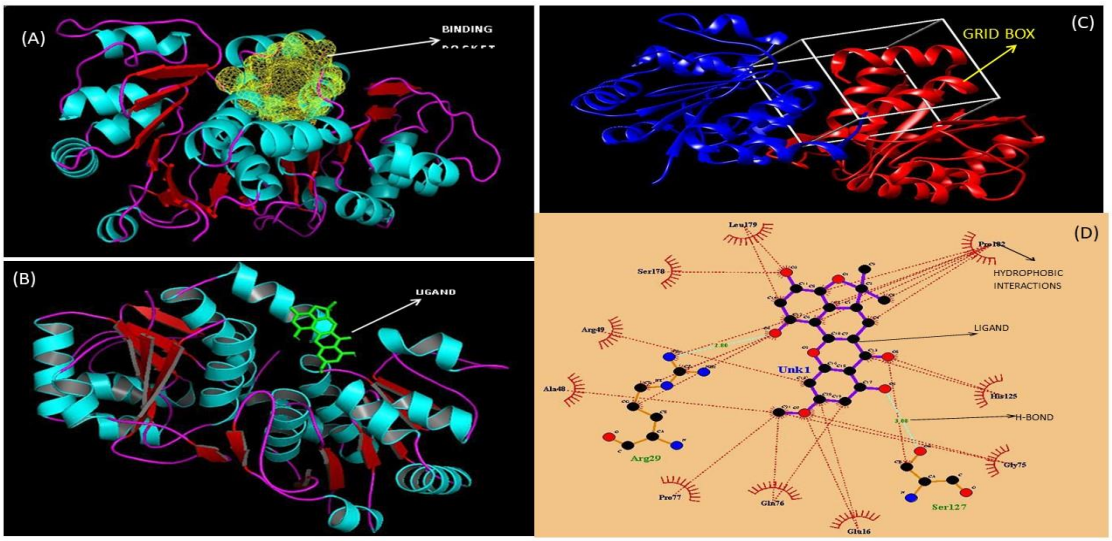


FIGURE 5 - (A) 3D structure of DJR-1 protein visualized in pymol software (B) Grid box formed around the binding site of DJR-1 protein, visualized in chimera software (C) Ligand Artonin K docked with DJR-1 protein (D) Ligplot representation of DJR-1 protein and Artonin K ligand with their interacting residues.

The analysis of the docking results with Dopamine D3 receptor of *C. elega*ns showed that, the ligand Artonin S had the highest binding energy of -7.9 kcal/mol and Artonin-K had the second highest binding energy of -7.5 kcal/mol. LigPlot+ analysis showed that, Artonin S had formed 3 H-bonds with the residues Leu160, Asn166, Ser169 and 9 hydrophobic interactions with the Trp73, Gln84, Tyr66, Tyr83, Asp83, Asp87, Ser91, Phe174, Ser170 and Phe165 dopamine D3 protein (FIGURE 6).

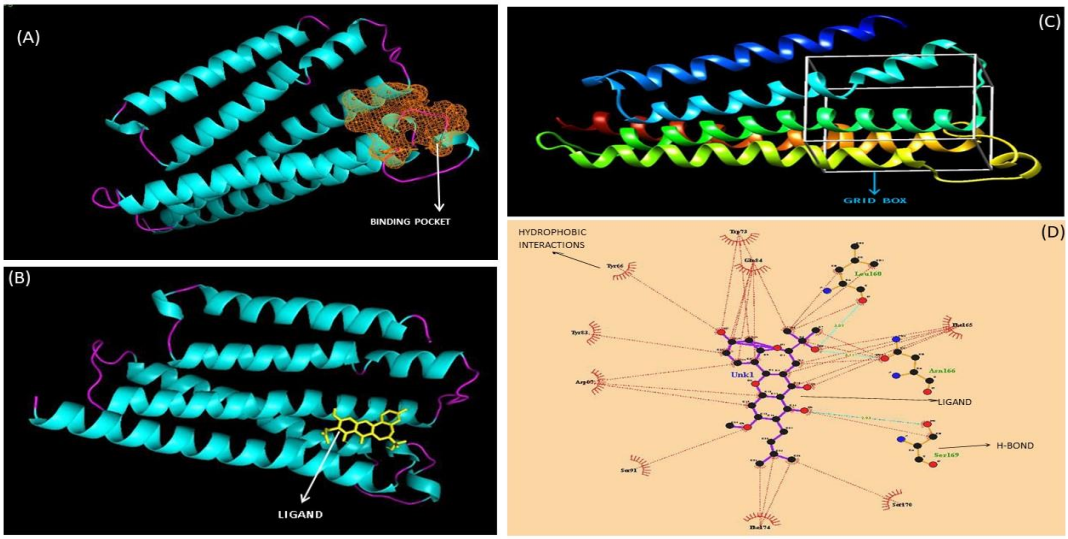


FIGURE 6 - (A) 3D structure of dopamine D3 protein visualized in Pymol software. (B) Grid box formed around the binding site of dopamine D3 protein,visualized in Chimera software (C) Ligand Artonin S docked with dopamine D3 protein (D) Ligplot representation of dopamine D3 protein and Artonin S ligand with their interacting residues.

The analysis of the docking result with SOD1 enzyme of *C. elega*ns showed that, the ligand Artonin S had the highest binding energy (lowest delta G value) of -8.7 kcal/mol and Artonin-K had the second highest binding energy of -7.9 kcal/mol. The number of H-bonds and hydrophobic interactions formed between the ligand and protein were analyzed using LigPlot+. Artonin-S had formed 7 H-bonds with the residues Asn53, Val54, Arg191, Glu146, Lys55 and 8 hydrophobic interactions with the residues Ile72, Pro156, Ile76, Ser75, Pro5, Trp78, Asp6 and Thr79 of the SOD-1 protein (FIGURE 7).

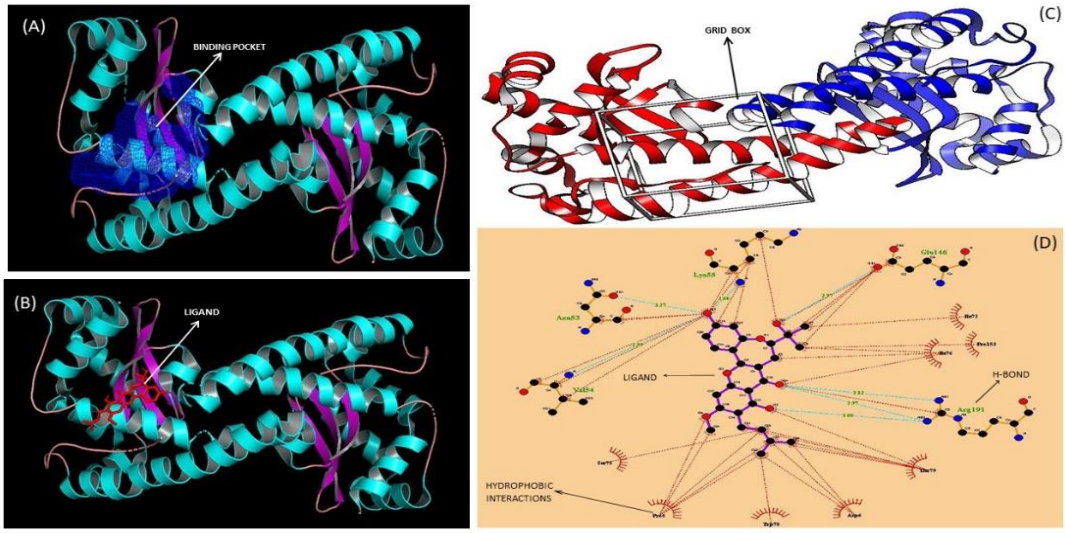


FIGURE 7 - (A) 3D structure of SOD-1 visualized in Pymol software (B) Grid box formed around the binding site of SOD-1 protein, visualized in Chimera software (C) Ligand Artonin S docked with SOD-1 protein (D) Ligplot representation of SOD-1 protein and Artonin S ligand with their interacting residues.

Receptor Ligand Interaction Analysis

Results obtained from interaction analysis of all ligands have proposed that a group of amino acid residues located on the binding cavity such as Val54, Lys55, Asn53, Glu146, and Arg191 in the target SOD-1 enzyme, Arg29, Ser178, Ser127 and Arg49 in the target DJR-1 and Leu160, Asn166, Ser169 Glu57, Asp154, and His81 in the target Dopamine receptor D3 proteins act as a significant role in ligand binding (Leach et al., 2006). Best-ranked compounds Artonin K and Artonin S are docked deeply into the active site region forming

interactions with the residues Ser75, Leu179, Pro156, Gln76, and His125 (TABLE S3).

Analysis of Molecular Dynamic Simulation

Protein-ligand complexes with the highest binding affinity were attained from the result of docking and molecular dynamic simulation was done to study the stability of these protein-ligand complexes. It was carried out using Discovery Studio software using the CHAMM36 forcefield. The precipitous decent algorithm was pragmatic to energy minimization for 3000 cycle steps. Equilibration was performed at a simulation time of 400 ps, to relax the solvent in protein structure under constant temperature dynamic (NVT) conditions. The production simulation was done at a simulation time of 10000 ps under a constant temperature dynamic (NVT). The simulations were conducted at a constant temperature of 300 K, and snapshots of the molecular dynamics (MD) trajectories were saved at every 50 ps intervals for subsequent trajectory analysis. (Patel & Iii, 2004).

To delve deeper into the protein-ligand interaction, a molecular dynamics (MD) simulation of the SOD1-Artonin S complex was conducted. Trajectory analysis of the MD conformations involved calculating the root mean square deviation (RMSD) and root mean square fluctuation (RMSF). It was observed that the RMSD values for both the ligand and protein remained stable, ranging between 36 nm and 38 nm. This stability suggests that the protein-ligand complex remained stable throughout the simulation. (FIGURE 8).

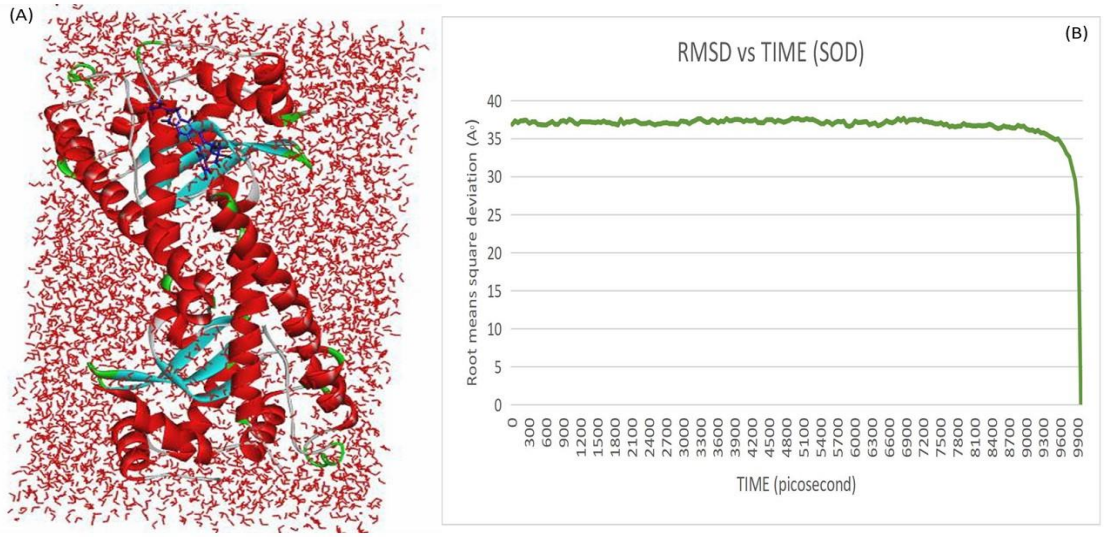


FIGURE 8 - Root mean square deviation (RMSD) of protein sidechain in molecular dynamics simulation. true value of rmsd for a simulation time of 10000 ps. (A) md simulation of sod1-artonin s complex (B) rmsd graph of sod1-artonin s complex.

MD simulation of dopamine D3 – Artonin S complex was done and Trajectory analysis of MD conformations was calculated for root mean square deviation (RMSD) and root mean square fluctuation (RMSF). It was observed that the root means standard deviation (RMSD) of the protein and ligand was stable between 31 nm and 33 nm, which indicated that the protein-ligand complex was stable (FIGURE 9).

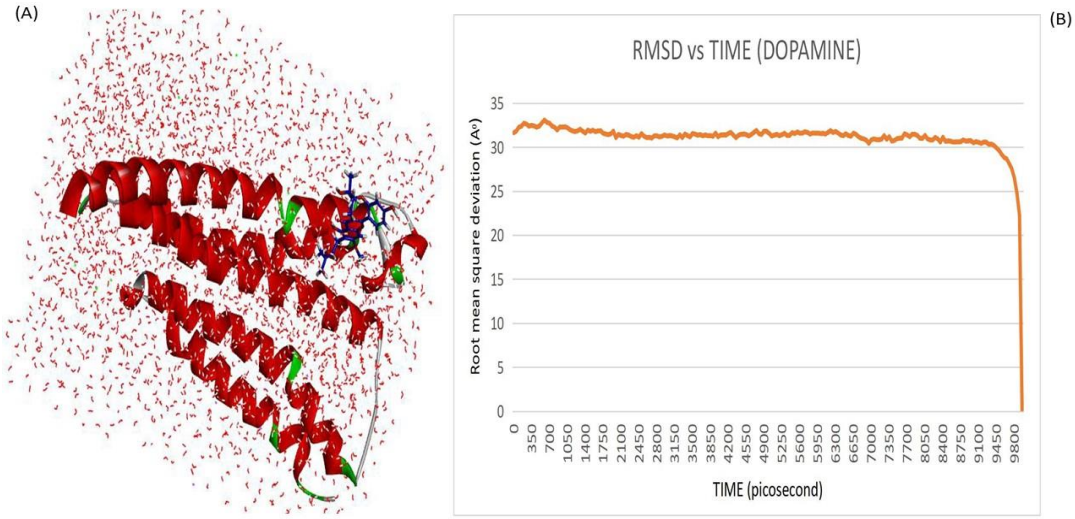


FIGURE 9 - Root mean square deviation (RMSD) of protein sidechain in molecular dynamics simulation. the true value of rmsd for a simulation time of 10000 ps. (A) MD simulation of dopamine d3-artonin s complex (B) rmsd graph of dopamine d3-artonin s complex.

A molecular dynamics simulation of the DJR1-Artonin K complex was performed, and trajectory analysis was conducted to calculate the root mean square deviation (RMSD) and root mean square fluctuation (RMSF). The RMSD of the ligand-protein complex remained stable, ranging between 29 nm and 31 nm. This observation indicates that the protein-ligand complex maintained stability throughout the simulation (FIGURE 10).

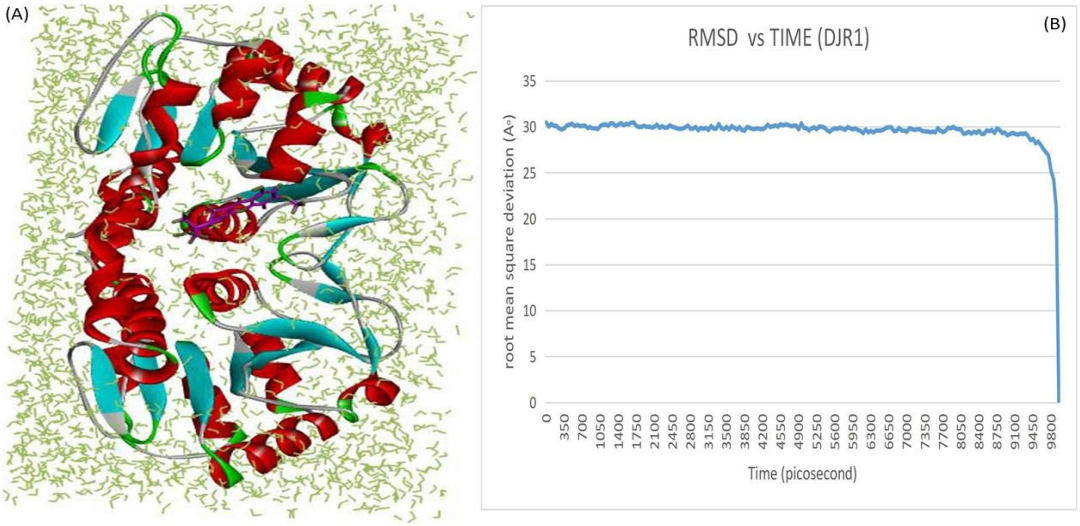


FIGURE 10 - Root Mean Square Deviation (RMSD) of protein sidechain in molecular dynamics simulation. The true value of RMSD for a simulation time of 10000 ps. (A) md simulation of djr1-artonin k complex (B) rmsd graph of djr1-artonin k complex.

In this *in silico* work, a new model of the DJR-1 protein and Dopamine D3 receptor of *C. elegans* was generated using homology modeling and validation studies confirmed that the predicted structures are reliable. The interactions between bioactive molecules of *Artocarpus heterophyllus* and SOD-1 enzyme, DJR-1 protein, and dopamine D3 receptor were studied using computation methods. *The in-silico* method applied in this study aided in identifying the ligands for the dealing with neurodegenerative Parkinson's disease by reducing oxidative stress. This approach reduces the time and cost involved in developing a drug and analyzing the likelihood of the drug prior to clinical trials.

Based on binding energy and hydrogen bonds formed between the protein and ligands docking results were analyzed. The results were compared to find out the best ligand which can

activate the properties of the protein. The study evinces that, the compounds of *Artocarpus heterophyllus* Artonin K and Artonin S showed the best docking results with SOD-1, DJR-1 protein, and dopamine D3 receptor. The binding energy of Artonin S was found to be -8.7 Kcal/mol, -7.2 Kcal/mol, and -7.9 Kcal/mol with SOD-1, DJR-1, and dopamine D3 receptors respectively. Likewise, the binding energy of Artonin K was found to be -7.9 Kcal/mol, -7.4 Kcal/mol, and -7.5 Kcal/mol with SOD-1, DJR-1, and dopamine D3 receptor respectively. The concept of binding energy says that the maximum the negative binding energy, the maximum will be the interaction. The theory behind this concept is that negative Delta G will have a spontaneous exothermic reaction, which means that the small molecule (ligand) will release energy after making a complex with protein and thus the protein-ligand complex becomes more stabilized than its native state. This is the reason by which it can be interpreted that the maximum release of energy will stabilize the docking complex.

The Molecular dynamic simulation of the complexes with the best docking result (SOD1-Artonin S complex, DJR-1 Artonin K complex, and Dopamine D3- Artonin S complex) was conducted to generate RMSD (rate mean square deviation) which also indicated that these protein-ligand interaction forms stable complexes. The ADMET studies also revealed the ability of Artonin K and Artonin S to act as non-mutagenic as well as non-carcinogenic with high gastrointestinal absorption, good bioavailability score, and minimal toxicity.

The overall results of this study impart that, these 2 bioactive compounds (Artonin K and Artonin S) might be effective as activators of the SOD enzyme and of E3 ligase activity of DJR-1 with agonists for the Dopamine D3 receptor. Therefore, they ought to be regarded as promising lead compounds for drug development targeting Parkinson's disease. The knowledge acquired in this study can be used in large-scale screening of neuroprotective biomolecules and can be further implemented in the design of effective treatments for PD.

IV. CONCLUSIONS

Docking scores and binding interactions revealed the selected phytochemicals were capable of binding protein targets (SOD1, DJR-1, and dopamine D3) and the best docking scores were shown by Artonin K and Artonin S. Thus, these can work as agonists for the proteins and elevating their activity. Molecular Dynamic simulation studies confirmed that the complex of Artonin K and Artonin S with SOD1, DJR-1, and Dopamine D3 were stable.

From the results of the current study, it can be concluded that, as phytochemicals Artonin K and Artonin S showed high binding energy against SOD-1 enzyme, DJR-1 protein, and dopamine D3 receptor when docked using AutoDock Vina and also possess a good ADMET profile, these compounds should be regarded as potential lead candidates for drug development targeting Parkinson's disease..

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