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Original Research Article

In Silico Molecular Docking Analysis of Selected Natural Biomolecules on Nitric Oxide Synthase

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ABSTRACT

Molecular docking has become essential for discovering new drugs from Natural Biomolecules. *Nitric Oxide, one of the paracrine hormones, is produced by a set of NO synthases (NOSs) from the precursor L-arginine.* Neuronal (nNOS), Inducible (iNOS), and Endothelial (eNOS) NO synthases are three different types of NOS. In this research, current study examined the molecular docking studies of selected biomolecules such as Rutin, Hesperidin, Morin, Luteolin, kaempferol, Myricetin, Apigenin, Curcumin, Cyanidin, quercetin, and Naringenin on Nitric Oxide Synthase. Docking Software (Discovery Studio 2.1, Docking algorithm-Libdock) to analyze the structure and prediction of selected natural biomolecules binding affinity for the targeted nitric oxide syntheses (NOS's) using Homology Modelling such as Primary structure analysis, secondary structure prediction, swiss model – 3D structure prediction, Model validation - PROCHECK, Ramachandran Plot analysis, Verify 3d, PROSA. The docking studies revealed the binding affinity of Rutin with the target protein Endothelial NOS and Neuronal NOS. Hesperidin shows affinity with Inducible NOS. Curcumin also showed high binding affinity with Neuronal and Inducible NOS. According to the findings, the natural biomolecules Rutin, Hesperidin, and Curcumin may be potential targets of nitric oxide synthase, a target for diabetic nephropathy, and exploited as a therapeutic option to evaluate experimentally via cell line research and *in vivo* studies to ensure its future viability.

Keywords: Docking, Biomolecules, Nitric oxide, Rutin, Hesperidin, Curcumin

Introduction

Molecular docking is a computational tool employed in the process of drug discovery. It is a technique that generates high-dimensional molecular structures. The current study used the Libdock score to find or detect the high binding affinity of the natural biomolecules. To help further investigate the potency of the natural biomolecules in Cell line research and *In vivo* studies. The docking input structural configuration is just as critical as docking, and analyzing the results of stochastic search techniques may sometimes confound.[1] The docking technique enables the description of a test molecule's behavior at a receptor target's binding site. A docking approach can determine the ligand's beneficial critical strength to the receptor complex with sufficient precision.[2] This research has led to the discovery of new active pharmaceutical ingredients that do not have many negative side effects.[3] Molecular docking is used to predict bioactive chemicals' binding affinity and determine the binding sites of bioactive chemicals and target proteins.[4]

Nitric Oxide

The gas nitric oxide (NO) is both volatile and lipophilic. It is found in almost all tissues and organs and acts as a free radical in various physiological and pathological circumstances. Nitric Oxide, one of the paracrine hormones, is produced by a set of NO synthases (NOSs) from the precursor L-arginine. *Neuronal (nNOS), Inducible (iNOS), and Endothelial (eNOS) NO synthases are three different types of NOS.*

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People with eNOS are born with 26 exons that comprise 21 kb of gene and on chromosome 7 (7q35-q36).[5] The role of estrogens in eNOS transcription regulation is currently under discussion. Both lipopolysaccharide and tumor necrosis factors affect the stability of eNOS messenger RNAs, hence inhibiting eNOS gene production.[6] Constantly expressed eNOS-mRNA is roughly 4052 nucleotides long and has a half-life of 10–35 hours.[7] As a result, protein synthesis is likely to persist for an extended period after the reduction of gene expression[7]. Additionally, senescent human endothelium cells exhibit reduced NO production and eNOS activity.[8]

NOS expression, specifically inducible nitric oxide synthases (iNOS), has been discovered in a range of human malignant tumors, including breast, lung, prostate, and bladder cancers, as well as colorectal and malignant melanoma.[9] Dermcidin (DCD), an 11 kDa protein, harms acute myocardial infarction by interfering with endothelial nitric oxide synthase. [10]

Endothelial dysfunction has to decouple the VEGF-nitric oxide axis, enhancing VEGF's pro-inflammatory and proliferation-promoting effects.[11] Vasodilators such as NO and prostacyclins inhibit endothelin-1. Endothelin-1 suppresses salt and water reabsorption by increasing vascular resistance in the kidney.[12] Consequently, blood flow, glomerular filtration rate, and salt and water reabsorption decreased.[13] It also promotes the development of glomerular cells and the creation of extracellular matrix.[14] Today, researchers are concentrating on applying their cutting-edge ideas to developing practical and low-risk treatment agents.[15] Secondary metabolites produced by medicinal plants offer effective therapeutic properties. In this study eleven natural biomolecules- Rutin, Hesperidin, Morin, Luteolin, Kaempferol, Myricetin, Apigenin, Curcumin, Cyanidin, Quercetin, and Naringenin were analysed for binding affinity with rat nNOS, iNOS, and eNOS (Table1).

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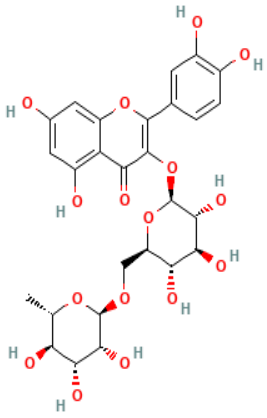
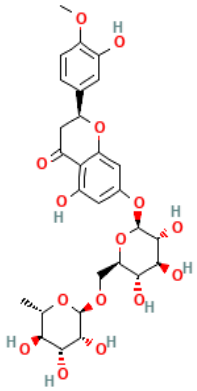
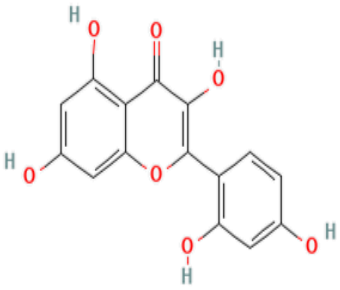
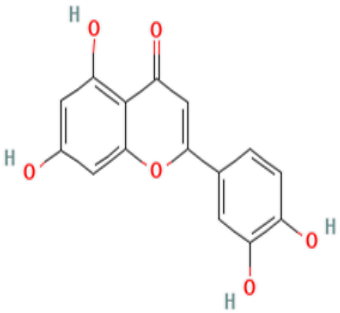
Table 1: About Selected Natural Biomolecules

Selected Natural Biomolecules	Chemical Structures of Biomolecules	Description	References
Rutin		Rutin: Rutin, a bioflavonoid contained in many foods, has shown promising anticancer properties, but its poor water solubility and pharmacokinetics restrict its use to very low dosages.	16
Hesperidin		Hesperidin is a glycosidic disaccharide formed from hesperetin and a position 6-O-(alpha-L-rhamnopyranosyl)-beta-D-glucopyranosyl. Pharmacological actions such as antihyperlipidemic, antihypertensive, cardioprotective, antidiabetic properties.	17
Morin		Morin is a flavonoid formed from the Moraceae family and a position of (3,5,7,2',4'-pentahydroxyflavone). Pharmacological activities such as diabetes, Parkinson's, cancer, cardiovascular, ischemia, and renal diseases .	18
Luteolin		Luteolin is a flavonoid polyphenol in various botanical groups in its aglycone and glycoside forms. Luteolin, also known as 3′, 4′,5,7-tetrahydroxyflavone, is an antioxidant and anti-inflammatory flavone found in green pepper, chamomile, celery, and parsley tea.	19

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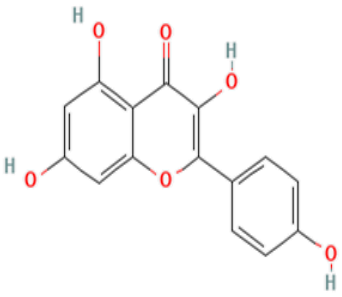
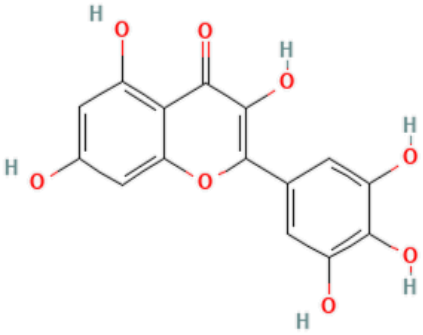
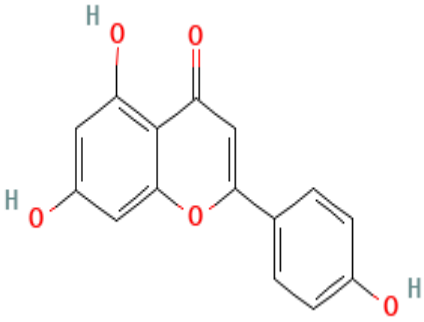
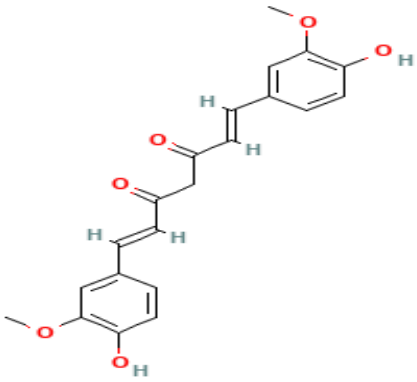
Kaempferol		Kaempferol is a tetrahydroxy flavone, which means that the four hydroxy groups are located on the flavone's carbon chain at positions 3, 5, 7, and 4'. Because of its antioxidant qualities, which include the decrease of oxidative stress, it is now being researched as a possible cancer therapy.	20
Myricetin		Myricetin is a hexahydroxy flavone, which is a flavone with hydroxy groups substituted at positions 3, 3', 4', 5, 5', and 7. It was isolated from the leaves of the shrub Myricarubra, among other places. It is an anticancer medication, antioxidant, plant metabolite, nutritional component, hypoglycaemic agent, and genoprotective agent.	17
Apigenin		Apigenin is a trihydroxyflavone with hydroxyl groups at positions 4', 5', and 7." Fruits (cherries, apples, grapes), vegetables (beans, broccoli, celery, onions, barley, tomatoes), herbs (endive), and liquids (tea, wine) all includes the flavonoid. It has significant anti-inflammatory, antibacterial, anti-inflammatory, and anti-cancer effects.	21
Curcumin		Curcumin is a 1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione. Curcuma longa and Curcuma domestica are turmeric plants; the rhizome is utilized as a spice (curry powder, also known as Indian saffron as well as yellow beetroot). It has significant anti-inflammatory, wound healing, anti-cancer, and anti-angiogenic effects.	22

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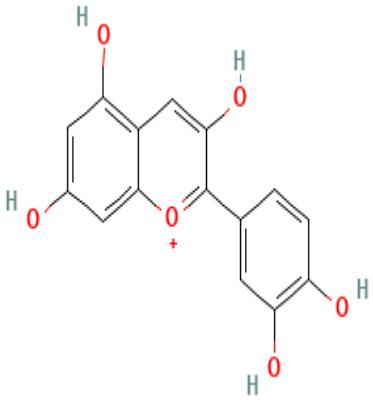
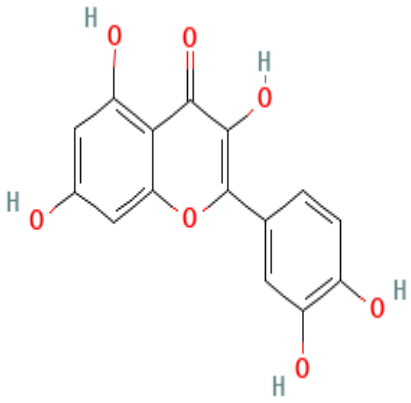
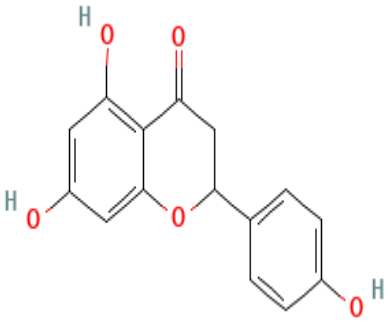
Cyanidin		Cyanidin is an anthocyanidin cation with hydroxy groups at positions 3, 3', 4', 5 and 7. It is a neuroprotective and antioxidant agent.	23
Quercetin		Despite its five hydroxy groups positioned at the 3-, 3'-, 4'-, 5-, and 7-positions, quercetin is a pentahydroxy flavone. It is a prevalent flavonoid in fruit, wine, and edible vegetables. It functions as an antioxidant, an antibacterial agent, a protein kinase inhibitor, and an antineoplastic agent.	24
Naringenin		Naringenin positioned at the (2s)-4',5,7-Trihydroxyflavan-4-one. It is the main flavanone in grapefruit and is present in many other fruits and plants. Pharmacological activities such as Alzheimer's, antifungal, antiviral and antibacterial activities.	25

Materials and Methods

Software and tools
Tools
Protparam - Primary Structure Analysis
PSIPRED - Secondary Structure Prediction
Swiss model - 3-Dimensional Structure Prediction
Model validation- PROCHECK, Ramachandran Plot analysis, Verify 3d, PROSA
Software
Discovery Studio 2.1

Docking algorithm-Libdock
Structural analysis of Specimen (Rat) Endothelial, Inducible, and Neuronal Nitric oxide synthase
Structural analysis retrieved the FASTA sequence of the specimen (Rat) Endothelial, Inducible, and Neuronal Nitric oxide synthase from the Swiss-prot database, which has a length of 1415 amino acids (Accession No: P29476). Primary and secondary structure analysis of Specimen (Rat) Endothelial, Inducible and Neuronal Nitric oxide synthase, physiochemical characterization, theoretical isoelectric point (pi), the total number of positive and negative residues and their extinction coefficients, the instability index, the half-life period, the aliphatic index, and the sample's grand average hydropathy (GRAVY) were all calculated using Expasy's Prot-Param server—secondary structural analysis using the PSI-PRED service.

Homology Modelling of Specimen (Rat) Endothelial, Inducible, and Neuronal Nitric Oxide Synthase and the Assessment of Model Accuracy
The stereochemical quality and accuracy of the Specimen (Rat) endothelial, inducible, and neuronal nitric oxide synthase models were determined using PROCHECK via SWISS-MODEL online servers. PROCHECK results in the form of Ramachandran plots, Verify3d, and a quality assessment performed by PROSA.

Protein Preparation
Specimen (Rat) Endothelial, Inducible, and Neuronal Nitric Oxide Synthase protein used Accelrys Discovery Studio 2.1. Using Discovery Studios, the protein chain was created by a clean protein process, which resolves problems like the lack of hydrogen atoms, missing atoms and residues, and the incorrect ordering of amino acid atoms. Using a pH of 7.4, protonate all residues and eliminate the

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water molecule from the mixture. To permit deprotonation of the glutamate and aspartate side chains while permitting protonation of the arginine and histidine side chains. The histidine side chains protonated in the presence of a proton donor, which is a very selective process. A CHARMm force field with a potential energy of -2065022208 kcal/mol, a Van der Waals energy of -3928640216 kcal/mol, and a first-order RMS (root-mean-square) gradient energy of 0.3928640216 kcal/mol. A sophisticated minimizer technique with a maximum number of steps of 1000 and a root mean square gradient of 0.1 was applied to reduce energy consumption. The protein complex brought up to the required grade of convergence gradient of 0.001 kcal/mol using a combination of steepest descent and conjugate gradient techniques.

Preparation of Ligand

Imported compounds are subjected to constraints such as the consistency of oxygen and nitrogen atoms' ionization states at physiological pH, hydrogen addition and deletion using the CHARMm force field, and finally, converted to 3D structures using Discovery Studio's (D. S) catalyst algorithm to create ligands. After that, the three-dimensional structures were exported in Protein Data Bank File format. These three-dimensional structures are as energy efficient as feasible using an intelligent minimizer strategy with a maximum number of steps of 1000 and an RMS gradient of 0.1, which continues by the steepest descent and conjugate gradient approach with an RMS gradient of 0.001 kcal mol^{-1}. The D.S. variable conformation synthesis module uses the Poling system to generate more distinct forms for each ligand. The FAST conformer technique creates a maximum of 255 configurations for each molecule to obtain a minimum 20 kcal/mol energy range above the global energy. This maximum number of conformations guarantees that the shape's interior space is entirely covered.

Identification of Active Site Pocket

The binding site of the simulated RAT Endothelial, Inducible, and Neuronal Nitric oxide synthase was defined and edited using Accelrys Discovery Studio 2.1's Define and Edit Binding Site tools and minimized the protein complex energy. The protein binding site in Discovery Studio, using the rubber method, and a sphere with a radius of 9 A° was constructed around the active site pocket.

Results and Discussion

Docking Studies

For accurate docking of compounds into the active site of the protein, molecular docking is Carried out through the LibDock module in Accelrys Discovery Studio 2.1. Primary and secondary structural analysis of Specimen (Rat) Endothelial, Inducible, and Neuronal Nitric oxide synthase was shown in Figures 1, 3, and 5.

Libdock is a high-throughput site-specific docking technique. The binding site features are called 'hotspots,' these site spheres are resolved using the active site-fixed grid. The hotspot map of polar and polar clusters at the protein's active site is utilized to align the conformations of the interacting ligands with the protein's interaction sites, leading to significant interactions. Finally, a list of all reduced ligand postures and the ligand scores corresponding to each stance returned—the standard pair-wise procedure utilized to investigate each site based on the Libdock score. The ligand with the highest Libdock score calculates the protein-ligand complex binding energies. To perform the critical mode of analysis on the challenging posture with the highest acute point.

In the current investigation, the selected natural biomolecules Rutin, Hesperidin, Morin, Luteolin, Kaempferol, Myricetin, Apigenin, Curcumin, Cyanidin, Quercetin, and Naringenin docked against the designated target proteins eNOS, iNOS, and nNOS. Hydrogen bond interactions of selected natural biomolecules with Specimen (Rat) eNOS, iNOS, and nNOS: Green-dotted lines indicate hydrogen bond forms, whereas green letters indicate the amino acids involved in the bonding. The ball and stick model illustrates the biomolecules (carbon atoms represented in blue) for their predicted nitric oxide synthase affinity. (Figures 2, 4, and 6). The LibDock score helped to determine the mods and affinities of chosen natural biomolecules with a rat (specimen) eNOS, iNOS, and nNOS model. The LibDock score and amino acid residues defined the ligand shapes in (TABLE 2). Based on the results of natural biomolecules by LibDock scoring pattern, Rutin reported the highest LibDock score amongst other biomolecules in models like eNOS with 158.602 kcal/mol and nNOS with 119.72 kcal/mol. Hesperidin LibDock score with 152.184 kcal/mol is higher than other biomolecules in the iNOS Model. Curcumin shows good binding affinity with LibDock score 141.611 kcal/mol in the iNOS model. Hesperidin reported a good LibDock score amongst other biomolecules in models like eNOS with 150.977 kcal/mol and nNOS with 112.331 Kcal/Mol. Curcumin Libdock Score of 106.604 Kcal/Mol is higher than other biomolecules in the nNOS model. The above study results helped to continue the *In Vitro* and *In Vivo* studies.

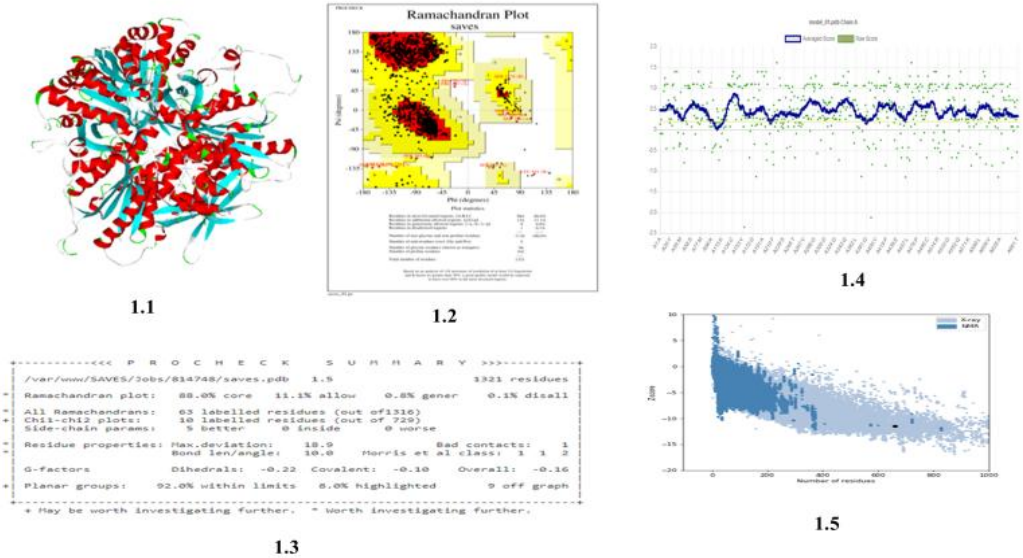

Figure 1: 1.1) Three-dimensional Structure Prediction by Swiss model, 1.2) Model validation: Ramachandran plot, 1.3) Procheck, 1.4) Verify 3D, 1.5) Prosa, Overall model quality, Z-Score: -11.47

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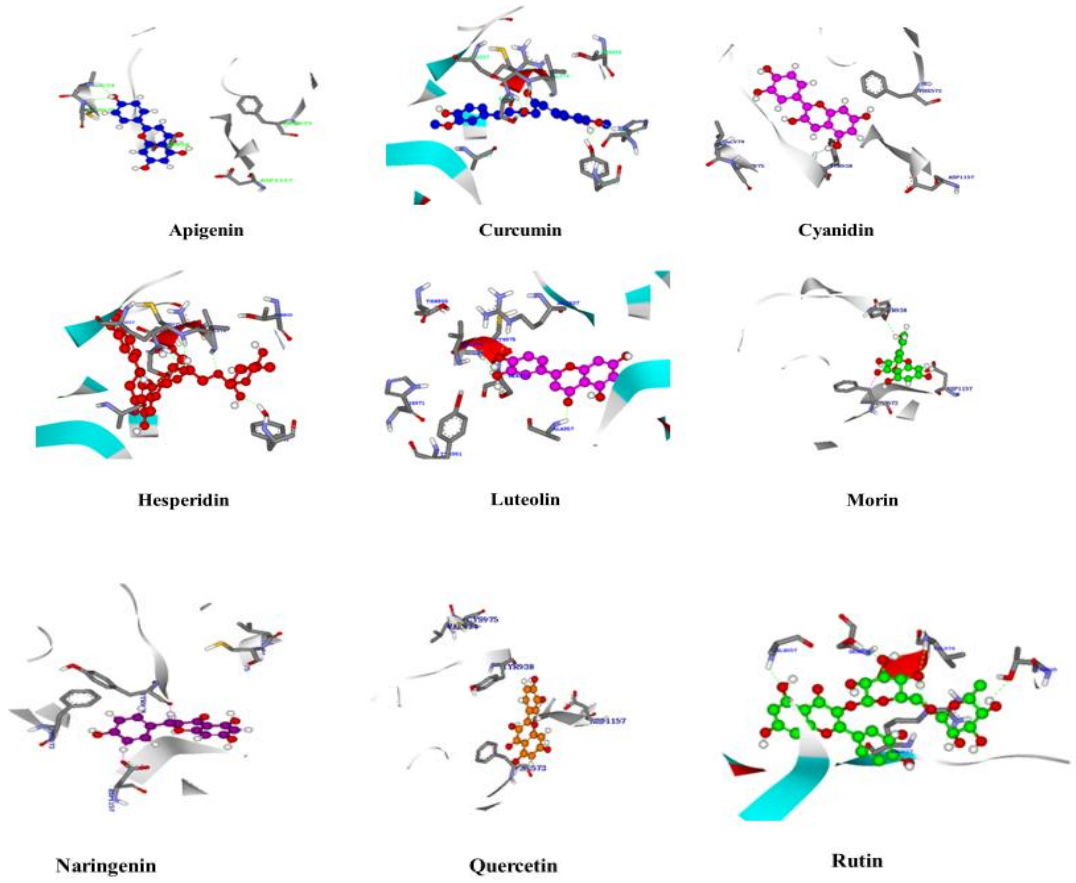

Figure 2: Hydrogen bond interactions of selected natural biomolecules with Specimen (Rat) endothelial nitric oxide synthase (Homology Modelling: Protein 3D structure from its amino acid sequence) Green-dotted lines indicate hydrogen bond forms, whereas green letters indicate the amino acids involved in the bonding. The ball and stick model illustrates the biomolecules (carbon atoms represented in blue).

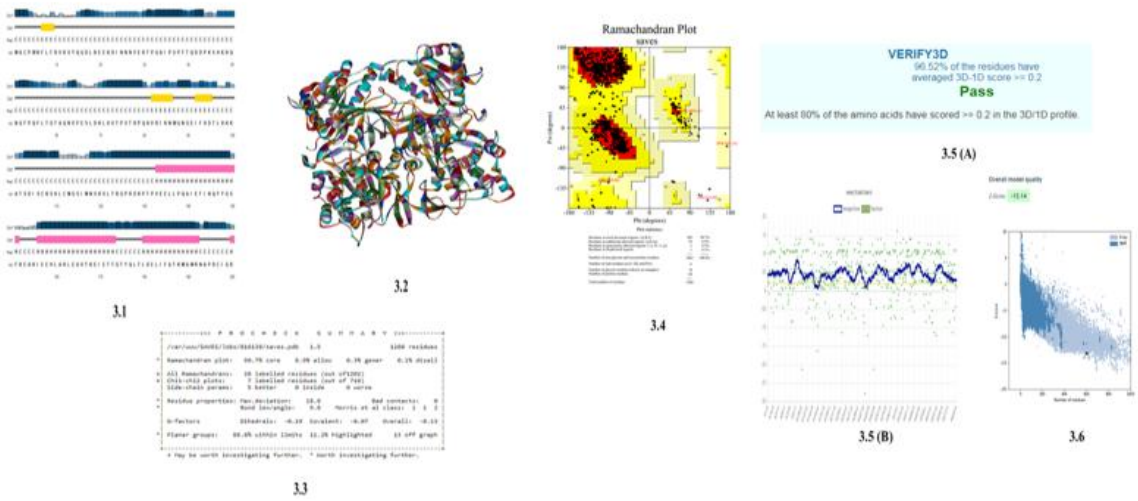

Figure 3: 3.1) Secondary Structure prediction done by SOPMA, 3.2) Three-dimensional Structure prediction done by using Swiss model, 3.3) Model validation done by PROCHECK, 3.4) Ramachandran Plot analysis, 3.5) A&B - Verify 3D, 3.6) PROSA results.

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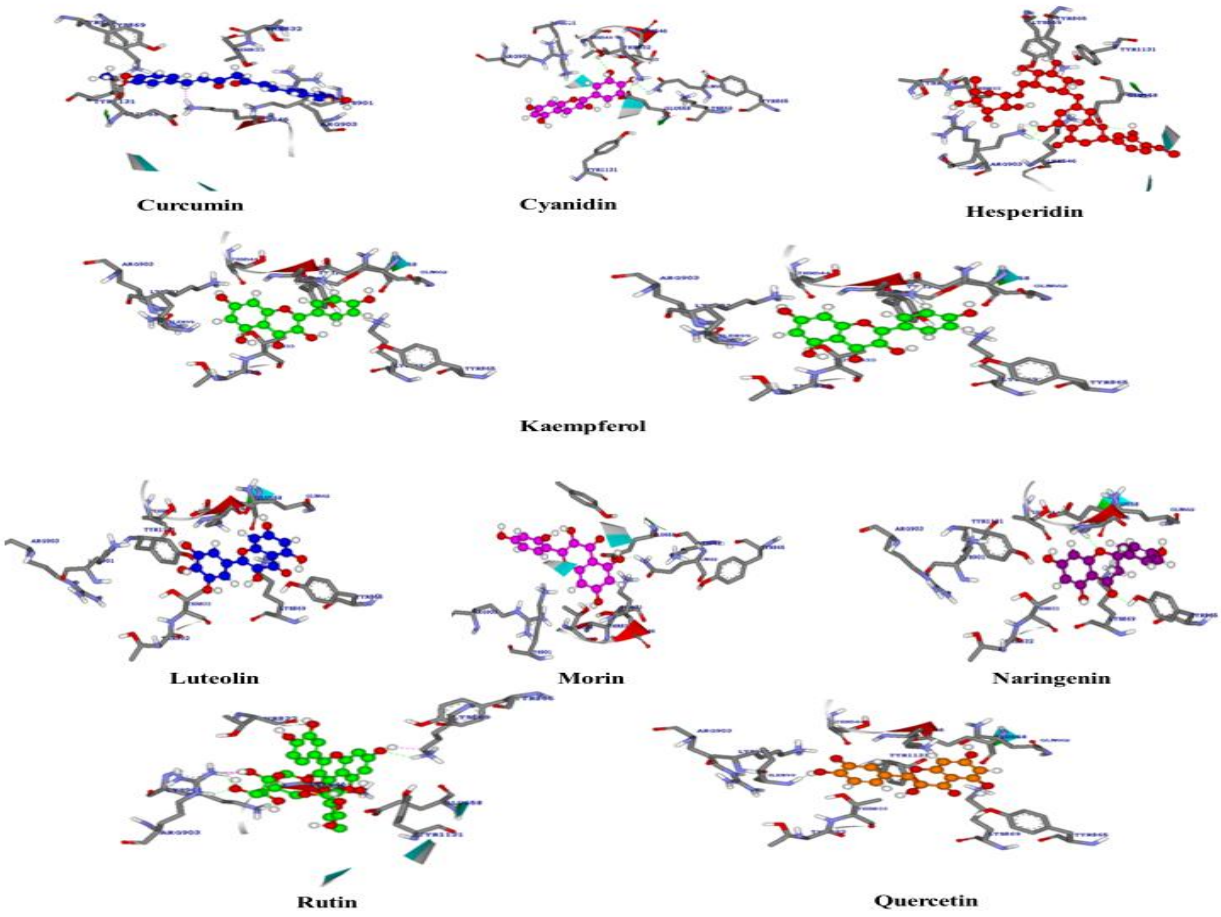
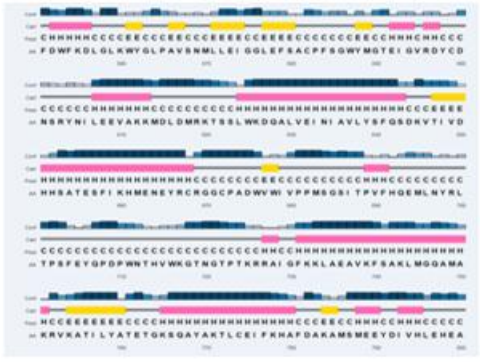
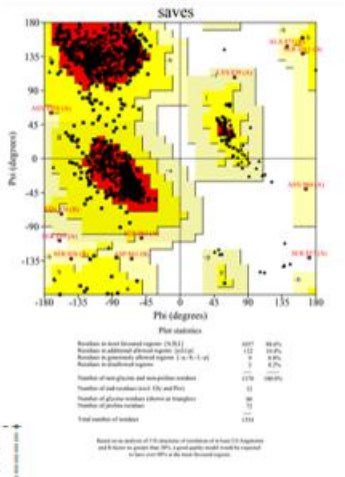


Figure 4: Hydrogen bond interactions of selected natural biomolecules with Specimen (Rat) inducible nitric oxide synthase (Homology Modelling: Protein 3D structure from its amino acid sequence) Green-dotted lines indicate hydrogen bond forms, whereas green letters indicate the amino acids involved in the bonding. The ball and stick model illustrates the biomolecules (carbon atoms represented in blue).



5.1

5.2



5.3

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+----------<<< P R O C H E C K    S U M M A R Y >>>----------+
|  /var/www/SAVES/Jobs/8144l4/saves.pdb   1.5                 1334 residues
|
*|  Ramachandran plot:    88.6% core    10.4% allow    0.8% gener    0.2% disall
|
-|  All Ramachandrans:    41 labelled residues (out of1322)
+|  Chi1-chi2 plots:      13 labelled residues (out of 809)
|  Side-chain params:     5 better      0 inside      0 worse
|
-|  Residue properties: Max.deviation:    18.7          Bad contacts:     4
-|                      Bond len/angle:   13.8    Morris et al class:   1   2   2
|
|  G-factors           Dihedrals:  -0.23  Covalent:   0.03    Overall:  -0.11
|
+|  Planar groups:    92.0% within limits    8.0% highlighted       3 off graph
+----------------------------------------------------------------------+
  + May be worth investigating further.  * Worth investigating further.
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5.4

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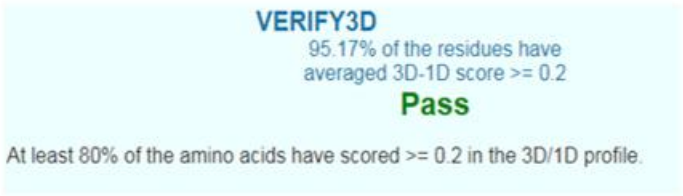
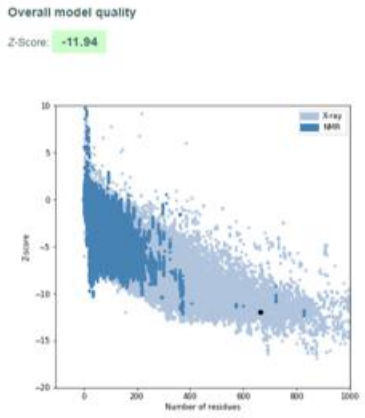


5.5 (A)

5.5 (B)



6.6

Figure 5: 5.1) Secondary Structure prediction done by PSIPRED, 5.2) Three-dimensional structure prediction using Swiss model, 5.3) Model validation: Ramachandran Plot analysis, 5.4) Procheck, 5.5) A & B – Verify 3D, 5.6) Prosa.

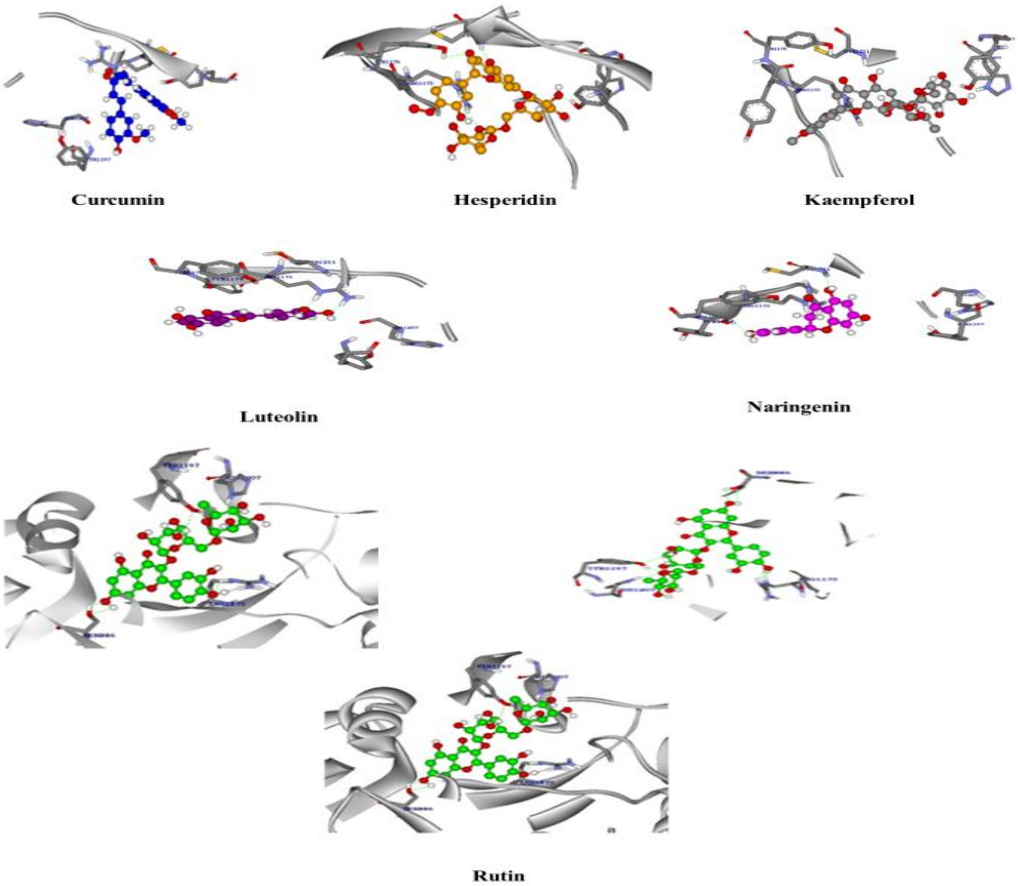


Figure 6: Hydrogen bond interactions of selected natural biomolecules with Specimen (Rat) neuronal nitric oxide synthase (Homology Modelling: Protein 3D structure from its amino acid sequence) Several types of hydrogen bonds are depicted by green-dotted lines and green letters, which represent the amino acids that are involved in the bonding process. The biomolecules are simulated using a ball and stick arrangement to illustrate their structure (carbon atoms are represented in blue colour).

Table 2: Targeted selected natural biomolecules with endothelial, inducible, and neuronal nitric oxide syntheses Libdock scores ranking

Natural Biomolecules	eNOS LibDock Score	Natural Biomolecules	iNOS LibDock Score	Natural Biomolecules	nNOS LibDock Score
Rutin	158.602	Hesperidin	152.184	Rutin	119.72
Hesperidin	150.977	Curcumin	141.611	Hesperidin	112.331
Morin	120.439	Rutin	119.508	Curcumin	106.604
Luteolin	119.656	Cyanidin	114.733	Naringenin	79.743
Kaempferol	113.104	Luteolin	107.152	Luteolin	82.069
Myricetin	106.965	Morin	104.074	Kaempferol	84.869
Apigenin	106.505	Apigenin	100.818		
Curcumin	105.345	Naringenin	100.242		
Cyanidin	97.875	Quercetin	90.988		
Quercetin	95.188	Kaempferol	86.051		
Naringenin	82.386	Morin	104.074		

Conclusion

According to the findings, the natural biomolecules Rutin, Hesperidin, and Curcumin may be potential targets of nitric oxide synthase, a target for diabetic nephropathy, and exploited as a therapeutic option to evaluate experimentally via cell line research and *in vivo* studies to ensure its future viability.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

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