

IN-SILICO DRUG DISCOVERY OF POTENTIAL TARGETS IN NEPHROTIC SYNDROME

Submitted in partial fulfilment of the requirements for the award of Master of
Science in Bioinformatics and Data Science

By

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**DEPARTMENT OF BIOINFORMATICS AND DATA SCIENCE
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SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

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APRIL 2024



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DEPARTMENT OF BIOINFORMATICS AND DATA SCIENCE

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **TONUBHA BASAK (42739026)** who carried out the final year project entitled "**In-silico Drug Discovery for Potential Targets in Nephrotic Syndrome**" under our supervision from January 2024 to May 2024.

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DECLARATION

I, **TONUBHA BASAK (42739026)**, hereby declare that the Project Report titled **“In-Silico Drug discovery of potential targets in Nephrotic Syndrome”** done by me under the guidance of **Dr. Swetha Sunkar** is submitted in partial fulfilment for the award of Master of Science in Bioinformatics and Data Science.

DATE: 20.04.2024

PLACE: Chennai

A handwritten signature in black ink, appearing to read 'Tonubha Basak', with a horizontal line drawn underneath it.

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Nephrotic syndrome (NS) is a clinical entity characterised by heavy proteinuria, hypoproteinemia and edema. Nephrotic syndrome is caused by acquired and genetic defects of the kidney filtration barrier located in the glomerular capillary wall. Nephrotic syndrome is a heterogeneous disease caused by the mutation of several genes out of which NPHS1 and NPHS2 are studied here. Homozygous or compound heterozygous mutations in NPHS1 and NPHS2 continue to be the cause of early onset of non-syndromic nephrotic syndrome (Steroid-resistant nephrotic syndrome) NPHS1 encodes for podocin protein and is involved in causing nephrotic syndrome type 1 and NPHS2 encodes for nephrin protein and is involved in causing the nephrotic syndrome type 2. The mutations in podocin (D160G, V180M and R291W) and nephrin (L376V and P368S) in the particular domains of the studied proteins are associated with the pathogenesis of nephrotic syndrome. Molecular docking studies were carried out by Biovia Discovery Studio to understand and analyse the ligand-receptor interaction between the wild and mutant type of the potential targets with the computationally filtered phytochemicals retrieved from *Paederia foetida*, *Kalanchoe pinnata*, *Boerhavia diffusa* plants. We modelled the 3D structure of the podocin by SWISS MODEL and validated via MOLPROBITY, Discovery studio and PROCHECK to get the Ramachandran plot, clash score, molprobability score and more stabilised structure of the protein structures. This study reveals the probable drug compounds for potential targets in nephrotic syndrome. The recognition of the probable phytochemicals and their effects by computational analysis and study can facilitate the drug discovery and development process for nephrotic syndrome. As there is a confined number of available drugs for the treatment of patients suffering from nephrotic syndrome, this study could provide new ideas for the research on the in-silico approach of drug discovery for nephrotic syndrome.

Keywords: Nephrotic syndrome, Mutation, NPHS1, NPHS2, *Paederia foetida*, *Boerhavia diffusa*, *Kalanchoe pinnata*, Molecular docking.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPANSION
NS	Nephrotic Syndrome
CNS	Congenital Nephrotic Syndrome
NPHS1	NePHrotic Syndrome 1
NPHS2	NePHrotic Syndrome 2
CADD	Computer-Aided Drug Design
LBDD	Ligand-Based Drug Discovery
SBDD	Structure-Based Drug Discovery
NVT	Number of particles-Volume Temperature
RMSD	Root-mean Square Deviation
P368S	Proline368Serine
L376V	Leucine376Valine
R138Q	Arginine138Glutamine
D160G	Aspartate160Glycine
V180M	Valine180Methionine

K	Kelvin
MD	Molecular Dynamics
IMPPAT	Indian Medicinal Plant, Phytochemicals, And Therapeutics
ADME	Absorption, Distribution, Metabolism and Excretion
TOPKAT	TOxicity Prediction by Komputer Assisted Technology
SMILES	Simplified Molecular Input Line Entry System
PDB	Protein Data Bank
NCBI	National Centre for Biotechnology Information
OMIM	Online Mendelian Inheritance in Man
SPFH_Domain	Stomatin/prohibitin/flotillin/HflKC_Dom ain
SIFT	Sorting Intolerant From Tolerant
SNP	Single Nucleotide Polymorphism

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CHAPTER 1

INTRODUCTION

1.1. *NEPHROTIC SYNDROME (IDIOPATHIC)*

Nephrotic Syndrome (NS) is a renal disease which is traced by immense proteinuria, hypoalbuminemia, edema and hyperlipidemia. Proteinuria is a secondary concern of this glomerular filtration barrier defect, infections and thromboembolic events are the major complications of nephrotic syndrome (Niaudet, 2022). Nephrotic syndrome can be caused by both acquired and genetic disorders of the filtration barrier of the kidney in the glomerular capillary wall. The kidney filtration barrier comprises three layers: 1. The fenestrated endothelium, 2. The glomerular basement membrane (GBM), 3. The epithelial cell or the podocytes along with the distal foot processes and interposed slit diaphragms (SDs) (Jalanko, et al., 2023).

The glomerular filtration barrier acts like a sieve in our kidney which prevents unnecessary passage of protein molecules through the barrier and prevents its loss into the urine. Nephrin and podocin are two major proteins of the podocytes of the kidney, present on the slit diaphragms (SDs) of the glomerular filtration barrier (Jalanko, et al., 2023). The gene NPHS2 encodes a protein that plays a role in the regulation of glomerular permeability. Mutations in this gene cause steroid-resistant nephrotic syndrome. Alternative splicing results in multiple transcript variants. Podocin has a hairpin-like structure which belongs to the stomatin family (Jalanko, et al., 2023 and Tabassum, et al., 2014). The gene NPSH1 encodes a member of the immunoglobulin family of cell adhesion molecules that functions in the glomerular filtration barrier in the kidney. The gene is primarily expressed in renal tissues, and the protein is a type-1 transmembrane protein found at the slit diaphragm of glomerular podocytes. The slit diaphragm is thought to function as an ultrafilter to exclude albumin and other plasma macromolecules in the formation of urine. Mutations in this gene result in Finnish-type congenital nephrosis 1, characterised by severe

proteinuria and loss of the slit diaphragm and foot processes (Sahu, et al., 2020).

The mutations in nephrin and podocin can cause nephrotic syndrome (Sahu, et al., 2020). Genetic alterations in NPHS1 are rare to be manifested in later phases of life. However, people affected by NPHS1 mutations have been found to have the onset from the range of age 6 months to 8 years. Mutations in the NPHS2 gene can cause nephrotic syndrome at any age and more than 100 mutations have been reported in patients with autosomal-recessive SNRS (Steroid- resistant nephrotic syndrome) (Jalanko, et al., 2023).. The domains of podocin and nephrin that are most prone to mutations are reported to be the SPFH_PODOCIN domain and Ig4-like domain respectively (Gbadegesin, et al., 2022, Tabassum, et al., 2014 and Sahu, et al., 2022).

Nephrotic syndrome is a type of common renal disorder found in children and adults. Historically, Roelans gave the first clinical description of nephrotic syndrome in the late fifteenth century, whereas Zuinger at a later aspect of life provided a detailed description of this disease and its importance as a result of chronic kidney failure in pre pre-steroid era. The nephrotic syndrome has been observed to be responsive to corticosteroid therapy in some cases and it is characterised with the remission and relapse of the disease which led to many further observations that are highly useful in the treatment procedure and the prognosis of the disease. About approximately 80% of the children diagnosed with idiopathic nephrotic syndrome respond to corticosteroid therapy with complete resolution of proteinuria and edema. In the steroid-responsive group of patients, the disease development is variable, with about 60% of the patients having frequent relapses or being dependent on steroids for the control in remission period (Gbadegesin, et al., 2008).

**THE
INTERN
AT WORK**

- proteinuria $\geq 3\text{g/day}$
- hypoalbuminemia ($<25\text{ g/L}$)
- peripheral edema
- hyperlipidemia

- 1°
 - minimal change disease (MCD)
 - focal segmental glomerulosclerosis (FSGS)
 - membranous nephropathy (MN)
- 2°
 - diabetes mellitus
 - infectious
 - FSGS: parvovirus, HIV
 - MN: hepatitis B, C
 - malignant
 - MCD: hematologic malignancies
 - MN: solid organ tumours
 - light chain disease (amyloidosis, myeloma)
 - autoimmune: MN: lupus nephritis class V
 - meds
 - FSGS: lithium, IFN, bisphosphonates
 - MCD: NSAIDs
 - hyperfiltration: FSGS: obesity, congenital uro. abn.
 - pregnancy (pre-eclampsia)

- 1) Na/H₂O retention
- 2) proteinuria
- 3) hyperlipidemia
- 4) hypercoagulability
- 5) infection risk
- 6) kidney function loss

urine dipstick ↓ urine ACR/PCR ≥ 300 mg/mmol ↓ 24-hr urine protein ≥ 3 g/day	(+) serum: albumin lipid panel creatinine lytes
kidney biopsy	

- **Pitting edema**
 - distribution of edema
 - weight changes
 - chronicity
- **Dyspnea**
 - pleural effusions?
 - signs of VTE: calf pain, pleuritic chest pain, hemoptysis
- **Hypertension**
- **Skin changes:** rashes, xanthomas, leukonychia
- **Lymphadenopathy**
- **Lymph exam**

1*: immunosuppression (steroids, MMF, rituximab, CNIs) guided by degree of proteinuria
2*: targeted at underlying cause

For all: conservative management targeting the complications...

- 1) **Na/H₂O retention**: fluid restrict to 1.5L/day, Na restrict to 2g/day, diuretics prn
- 2) **proteinuria**: ACEi/ARB +/- SGLT2i (to ↓ intraglomerular pressure)
- 3) **hyperlipidemia**: target LDL < 2.0 or ↓ 50% from baseline
- 4) **hypercoagulability**: prophylactic anticoagulation if albumin <20-25 or pregnant
- 5) **infection risk**: keep vaccines up to date

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Congenital nephrotic syndrome (CNS) or the Nephrotic syndrome type 2 is a form of nephrotic syndrome that occurs in the life of a patient after the first 3 months of life. It is idiopathic which occurs at the later point of life caused by the several gene mutations (Jalanko et al., 2022).

1.3.TYPES AND TERMS IN NEPHROTIC SYNDROME

On the basis of this finding, some clinical definitions should be established to clarify the patient responses during treatment.

Nephrotic syndrome: The identification of nephrotic syndrome requires the presence of intense proteinuria (>40 mg/m²/hr or a urine protein/creatinine ratio >2.0 mg/mg), edema and hypoalbuminemia (<2.5 g/dl) (Niaudet, 2004 and The Journal of pediatrics, 1981).

Remission: It is defined by a noticeable decline in the proteinuria (to <4 mg/m²/hr or urine albumin dipstick of 0) accompanied by the reduction of edema and normalisation of serum albumin 3.5g/dl (Niaudet, 2004 and The Journal of pediatrics, 1981).

Relapse: This condition is characterised by the reappearance of the intense proteinuria along with the acquaintance of edema (>40 mg/m²/hr, urine protein/creatinine ratio >2.0 mg/mg, or urine albumin dipstick $\geq 2+$ on 3 consecutive days) (Niaudet, 2004 and The Journal of pediatrics, 1981).

Steroid-Sensitive Nephrotic Syndrome (SSNS): Patients who are responsive to the corticosteroid therapy and enter the remission phase due to the exposure to the therapy are categorised into the Steroid- Sensitive Nephrotic Syndrome (SSNS) group of patients (Niaudet, 2004 and The Journal of pediatrics, 1981).

Steroid-Resistant Nephrotic Syndrome (SRNS): The group of patients who are unresponsive to the exposure of 8-week corticosteroid therapy are categorised Steroid- Resistant Nephrotic Syndrome (SRNS) patients (Niaudet, 2004 and The Journal of pediatrics, 1981).

Steroid-Dependent Nephrotic Syndrome (SDNS): The patients who are responsive to corticosteroid therapy and require a continuous low-dose treatment to maintain their remission period are said to have Steroid-Dependent Nephrotic Syndrome (SDNS) (Schulman, et al., 1988).

Frequent-Relapsing Nephrotic Syndrome (FRNS): Patients who enter complete remission and remain in it for several weeks after the exposure to corticosteroid therapy but experience repeated relapses of more than 4 times in

a duration of a 12-month period after the discontinuation of the treatment are considered to have Frequent Relapsing Nephrotic Syndrome (FRNS) (Schulman et al., 1988).

The groups of patients suffering from the conditions SDNS and FRNS are prone to develop complications of nephrotic syndrome along with which they also tend to develop complications as a result of the side effects due to the continuous exposure to steroids and other immunosuppressive agents. The patients suffering from these conditions also tend to develop several infections and physical disorders at a later phase of life (Gbadegesin et al., 2008).

1.4.MEDICINAL PLANTS IN NEPHROTIC SYNDROME

Boerhaviadiffusa, commonly known as Punarnava is a medicinal plant, popularly used to treat a variety of human disorders as mentioned in Ayurveda. The entire plant or some particular parts (aerial parts or roots) of the plant is used for its rich medicinal properties by the endemic and tribal people of India, Unani medicine in Arab countries and also the traditional Chinese medicine (Mahesh et al., 2012). *Paederiafoetida*, commonly known as "Gandhavadulia " has a variety of pharmacological and phytochemical importance. This whole plant has been used in ayurveda for the treatment of various diseases (Khushbu et al., 2010). *Kalanchoe pinnata*, a succulent perennial plant that is about 3m in height, is commonly known as "air plant" which has hollow stems, dark fleshy green leaves that are distinguishingly convoluted and trimmed in red. It is a medicinal plant greatly used for the treatment of kidney stones, gastric ulcer, pulmonary disorders, rheumatoid arthritis, etc. in the folk medicines (Quazi et al., 2011).

1.5. IN-SILICO DRUG DISCOVERY

The traditional way of drug discovery has always contained a lot of risk and has consumed a lot of money and time in executing the entire process of the drug discovery which includes target identification, validation, lead compound discovery, prediction, preclinical and clinical trials (Tang et. al, 2021). Recently, it has been estimated that the entire expenditure in the traditional way of drug discovery can go up to \$1.8 billion USD and the rate of its reduction in strength

is around 96% (Paul et. al, 2006). The reasons which are responsible for this reduction in rate includes the reduced drug absorption, distribution, metabolism, and excretion and toxicity (ADME-tox) (Giri and Bader, 2015). Generally, the in-vitro and in-vivo techniques are used to examine the safety of drugs including adverse effects and toxicity. Recent developments in the technology, such as organ-on-chip technology has advanced the ADME-Tox assessments greatly (Huh et al., 2011). Even so, these approaches remain expensive, time consuming and labour intensive. High-throughput screening (HTS) methodologies have been developed to facilitate the process of identification of pharmacologically active compounds from large numbers of molecules using automated assays (Misra et al., 2008).

1.6. COMPUTER AIDED-DRUG DESIGN (CADD)

Computer aided drug design is a combination of computational tools and techniques which are used to facilitate and elevate the process of discovering and designing new drugs. Recently, computer-aided drug design (CADD) approaches are receiving growing attention due to its ability to alleviate the time, labour, scale and cost related issues. CADD consists of the identification of potential targets for drugs through computational methodologies, virtual screening and filtration of huge chemical libraries for potential drug candidates, optimization of the potential chemical compounds and *in-silico* assessments of their potential toxic nature. After undergoing all the procedures, the potential drug candidate undergoes the *in vitro* or *in vivo* experiments required for the confirmation of the drug efficacy. Therefore, CADD approaches have proven to increase the success rate of drug discovery by narrowing down the number of chemical compounds to be examined and evaluated experimentally for their toxic and inefficient nature (Shaker et al., 2021).

There are several computer-aided drug design approaches that have been developed and combined with machine learning algorithms and techniques to enhance the efficiency and accuracy of CADD methods (Vamathevan et al., 2019). There are two CADD approaches that exist and are utilised based on the availability of information and data about the chemical compounds for the respective study, the two approaches are structure- based drug discovery

(SBDD) and ligand-based drug discovery (LBDD). When the experimental structural information about the target protein is available and a high-quality 3D structure can be generated, in this case the SBDD approach is taken into consideration. However, in the absence of experimental structural information through x-ray crystallography or nuclear magnetic resonance, the structure can be predicted using *in-silico* prediction methods such as homology modeling and *ab-initio* modeling. When the structure is not available and prediction is not possible, the LBDD approach is considered for the structure generation. However, in this case an earlier knowledge about the active chemical compounds of the structure should be present. Once the information and the 3D structure of the target protein is available, one can proceed for the further analysis and study with the aid of virtual screening and molecular docking (Kitchen et al., 2004).

1.7. TARGET IDENTIFICATION

A drug target is defined as a biological entity, usually a protein, that can regulate disease phenotypes (Katsila et al., 2016). Hence, the identification and determination of high-quality drug targets is the foremost and most important step in the drug discovery process. Traditionally, drug target identification approaches are carried out experimentally, like identifying and comparing differentially expressed genes in the diseased cells or tissues with the normal cells and tissues. Drug target identification also includes identifying protein compounds that are highly related or interconnected with the disease.

Traditional experimental strategies for target identification need molecular and biomolecular studies of disease pathophysiology. Even though these studies amplify and enhance our knowledge, these strategies can be very time consuming for searching promising drug candidates. Recently, there has been development of several advanced technologies which fasten and facilitate the process of target identification.

Experimental target identification can be pricey and are generally conducted at low-throughput scale due the complexity. To overcome these challenges, *in-silico* methods for the identification of the target protein have been developed

over the years of research and development. Target proteins can be computationally predicted with the help of experimental data, derived literature using text mining, or concluded from protein networks (Shaker et al., 2021).

Once a target has been identified, for further analysis, the effect of the target regulation on the disease phenotype needs to be confirmed (Drews, 2000). There are a number of methods for regulating the biological functions and examining the predicted targets and their effects on the phenotype of the disease.

1.8. *IN-SILICO SCREENING PROCESSES OF DRUG TARGETS*

The main aim performing drug discovery studies is to discover and find potential drug molecules which possess the ability to effect and modulate the function of an identified target protein and thereby modulate the phenotype of the disease. Also, it is necessary to identify small molecules which have good and high-quality pharmacokinetic properties and low toxic nature. Recent advances in the technology have created various new approaches like machine learning algorithms with high accuracy, accumulated knowledge bases and synthetic chemical compound libraries to virtually screen a large number of chemical compounds and rapidly evaluate their ADME-Tox properties.

1.9. *LIGAND-BASED DRUG DISCOVERY (LBDD)*

The ligand-based drug discovery approach uses previously acquired knowledge on active drugs such as the information about their structure, physical properties and chemical properties, to predict new drug compounds with similar biological effects (Fig. 1.). Prediction of the drug compounds is based on the similarity of features (e.g., aromaticity, hydrogen bond acceptors, hydrogen bond donors, hydrophobicity, anion, and cation residues) of the chemical compounds, assuming that the compounds sharing a high structural and physicochemical similarities are more likely to have similar biological activity (Martin et al., 2002). The ligand-based drug discovery approach is usually applied when the information about the structure of the target is not available.

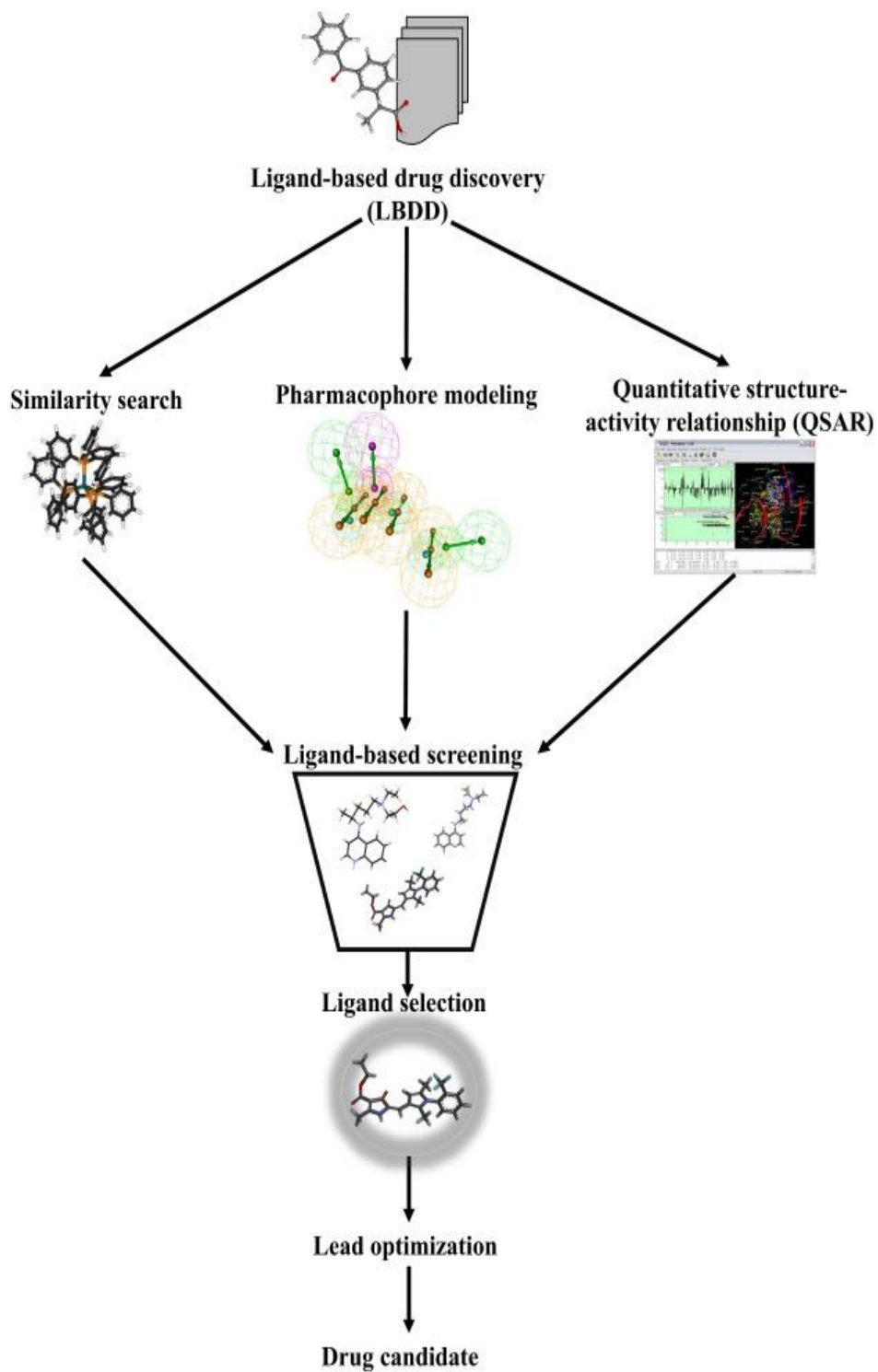


Fig:1.2: Ligand-based drug discovery workflow (Shaker et al., 2021).

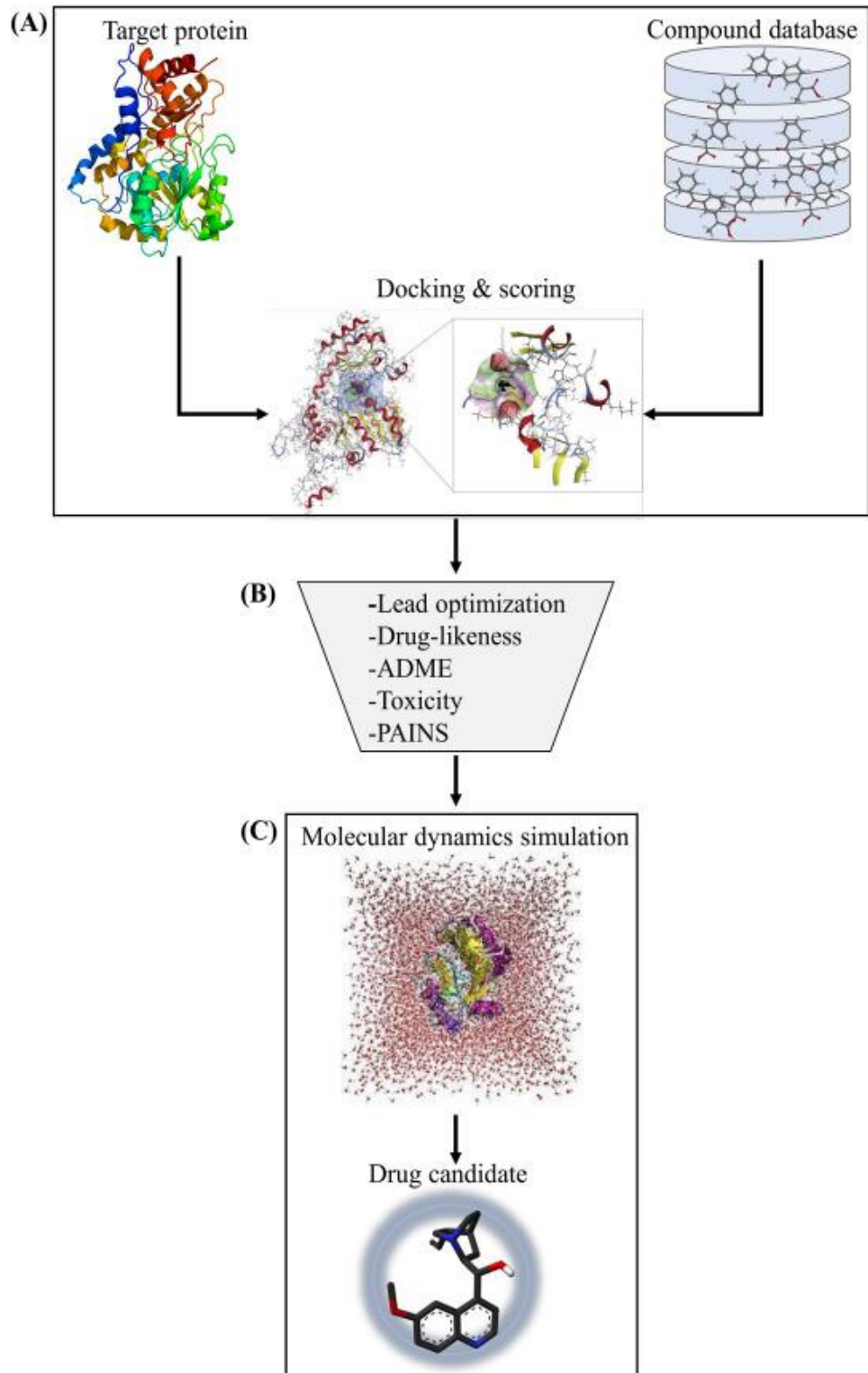


Fig:1.3: Structure-based drug discovery workflow (Shaker et al., 2021)

1.10. STRUCTURE-BASED DRUG DISCOVERY (SBDD)

In the structure-based drug discovery (SBDD) approach, unlike ligand-based drug discovery, calculate and measure the binding affinity between the target molecule, specifically a binding pocket and the chemical drug compounds (Rognan, 2010) (Fig. 2). In this approach of drug discovery, there are several steps that are included in the process like molecular docking, fragment-based docking, and molecular dynamic simulation to predict the binding affinity of the drugs with the target.

1.11. MOLECULAR DOCKING

Molecular docking is a step in the process of drug discovery which is implemented once the target protein and its 3D structure has been determined and finalised. With the use of molecular docking, the binding affinity of the ligand molecules with the target protein can be determined. The algorithms in molecular docking can help predict the preferred orientation and conformation of ligand molecules within the binding pocket of the target protein. This also helps calculate the binding affinity of the ligand molecules with the target protein by their electrostatic and van der Waals interactions (Combs, 2007, Coumar et al., 2009 and Gohlke &Khelbe, 2002). With the molecular docking algorithms, researchers can screen ligands virtually to find the ligands with the highest binding affinities. Docking methods can be divided into rigid and flexible docking methods where rigid docking considers only the fixed physicochemical or geometric complementarities and flexible docking methods deal with the flexibility between the ligands and the target (Halperin et al., 2002).

Molecular docking can also be used to study and predict the protein-protein interactions between the target and ligands and also evaluate the binding affinities of complexes. This aspect of the docking study assists to learn and provides a better understanding of the signalling pathways. Protein-protein interactions are also responsible for the cellular processes, hence learning and gaining insights about the protein-protein interaction through molecular docking studies can help scientists understand the functional mechanisms and their roles in the cells (Waugh, 1954).

1.12. MOLECULAR DYNAMIC SIMULATION.

The flexible nature of a protein is an important aspect to be considered in ligand binding, but predicting the movements and conformational changes of the protein binding pockets and ligands require a high computational cost due to the presence of complex atomic interactions between the ligand molecules and the protein target. This was a great challenge in history and to overcome this challenge, molecular dynamics (MD) simulation was introduced in the year 1970 (Karim et al., 2022). MD simulation helps determine the features of ligand-target complexes and their stability in drug discovery process and also facilitates the virtual screening of chemical compounds. It also facilitates the recognition and determination of additional druggable binding sites like allosteric sites and thus, leads to the design of more effective drug compounds (Grant et al., 2011 and Nair et al., 2012).

In computational drug discovery, molecular dynamic simulation is used to confirm the binding of the best docked complexes. The root mean square fluctuations, radius of gyration, the root mean square deviation and hydrogen bonding formations are used to analyse the stability of the complexes. For enhancing the performance of molecular dynamic simulation, a more accurate molecular force field is needed to perform the simulation of the movements of the atoms present in target proteins and ligands. Traditional MD simulations are based on two strategies, molecular mechanisms (MM) and quantum mechanism (QM) to perform more accurate simulation (Shaker et al., 2021).

LITERATURE SURVEY

2.1. *NEPHROTIC SYNDROME*

According to Bagga and Mantan, 2005, nephrotic syndrome is a significant chronic condition in children, predominantly characterized by minimal change disease. Research on its pathogenesis has highlighted the role of T lymphocyte dysregulation and vascular permeability factors, which can impact podocyte function and permselectivity. Although mutations in genes encoding essential podocyte proteins have been discovered, a comprehensive hypothesis explaining the pathogenesis remains elusive. Patients with nephrotic syndrome are vulnerable to severe infections and thromboembolic events. The long-term consequences of persistent hyperlipidemia and extended steroid use are becoming more apparent. Successful remission of proteinuria following corticosteroid treatment is a better predictor of long-term outcomes than renal histology. Prospective studies indicate that extending treatment duration for the initial episode leads to sustained remission and fewer relapses. Therapies such as levamisole, cyclophosphamide, cyclosporine, and mycophenolate mofetil benefit some patients with frequent relapses or steroid dependence. Managing steroid-resistant nephrotic syndrome is challenging; many patients who fail to achieve remission experience progressive renal damage. Calcineurin inhibitors (cyclosporine, tacrolimus) can induce remission in many patients but carry the risk of nephrotoxicity. Proteinuria reduction in children can also be achieved using angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers. Prospective trials are needed to establish effective and safe treatments for patients with frequent relapses, steroid dependence, and resistance.

In the article by Jalanko and Kääriäinen, 2023, nephrotic syndrome (NS) is a medical condition marked by significant proteinuria, low blood protein levels, and swelling. NS arises from acquired or genetic anomalies in the kidney filtration barrier found in the glomerular capillary wall. This barrier consists of

three layers: fenestrated endothelium, the glomerular basement membrane (GBM), and an epithelial cell (podocyte) layer with distal foot processes and slit diaphragms (SDs) in between. This filtration barrier acts as a molecular sieve that is selective in size and charge, effectively preventing plasma proteins from leaking into the urine. Genetic mutations affecting podocyte proteins result in proteinuria, and currently, 10-20% of sporadic and 30-40% of familial therapy-resistant NS (SRNS) cases are linked to a genetic defect.

2.2. CONGENITAL NEPHROTIC SYNDROME

In the article by Jalanko, 2009, congenital nephrotic syndrome (CNS) is an uncommon kidney condition marked by heavy proteinuria, low blood protein levels, and swelling that begins shortly after birth. Most cases result from genetic mutations in components of the glomerular filtration barrier, particularly nephrin and podocin. CNS can also be associated with a broader syndrome or be the result of a perinatal infection. Immunosuppressive treatment is ineffective in genetic forms of CNS, and kidney transplantation is the only definitive treatment. Before transplantation, managing these infants depends on the severity of proteinuria. In severe cases, daily albumin infusions are necessary to prevent dangerous edema. Treatment also includes a hypercaloric diet, thyroxin and mineral supplementation, prevention of blood clots, and quick response to infections. CNS patients without significant extrarenal symptoms have outcomes after kidney transplantation similar to other patient groups.

According to Kemper and Lemke, 2018, the outlook for congenital nephrotic syndrome (NS) has greatly improved in recent years. A recent registry report of 170 patients found no significant difference in mortality and transplant outcomes between Finnish and non-Finnish patients with NPHS1 mutations. Finnish patients began dialysis earlier due to early bilateral nephrectomy, while non-Finnish patients underwent various other interventions that were not specified in detail. Despite these variations, the outcomes of NPHS1 patients on renal replacement therapy were comparable to those of patients with congenital anomalies of the kidney and urinary tract (CAKUT). Details on treatment approaches and mortality prior to end-stage renal disease were not

provided. These factors, along with others, may affect morbidity and mortality in patients with NPHS1 and other genetic causes.

According to AbuMaziad et al., 2021, congenital nephrotic syndrome (CNS) is a complex type of nephrotic syndrome marked by severe proteinuria, low albumin levels, and swelling. A major characteristic of CNS is the substantial loss of plasma proteins. Diagnosis can occur in utero or within the first few weeks of life, usually before three months. CNS may arise from genetic or non-genetic causes, with pathogenic variants in genes such as NPHS1, NPHS2, LAMB2, WT1, and PLCE1 playing a role in the condition. The clinical progression of CNS can be complicated by severe edema, infections, thrombosis, hypothyroidism, and poor growth, among other issues. Treatment focuses on establishing vascular access, frequent intravenous albumin infusions, using diuretics, preventing infections, and providing nutritional support during the initial month of life. Kidney transplantation is the most effective therapy for these patients. CNS remains a challenge for clinicians in terms of diagnosis and management. This review aims to raise awareness about CNS's pathogenesis, diagnosis, and management.

2.3. IN-SILICO STUDY ON NEPHROTIC SYNDROME AND PHYTOCHEMICALS.

According to Tabassum et al., 2014, non-synonymous single nucleotide changes (nSNCs) are coding variants that result in amino acid substitutions in their corresponding proteins. These changes can impact protein function and are believed to have a greater effect on human health compared to single nucleotide changes in other parts of the genome. Sequence alterations can directly influence structural stability through conformational changes. When these changes occur near catalytic or active sites, they may alter protein function and consequently affect receptor-ligand complex interactions. This study examines the impact of human podocin mutations (G92C, P118L, R138Q, and D160G) on its structure. Podocin is a crucial glomerular integral membrane protein associated with steroid-resistant nephrotic syndrome. The protein has a hairpin-like structure consisting of 383 amino acids and serves as

a molecular link in a stretch-sensitive system, bearing homology to stomatin. We used Modeller to construct the 3D structure of podocin and validated it with PROCHECK, achieving a Ramachandran plot with 88.5% in the most favored region, as well as assessments of main chain, side chain, bad contacts, gauche, and pooled standard deviation. Further, the protein engineering tool Triton was employed to induce mutagenesis corresponding to the four variants G92C, P118L, R138Q, and D160G in the wild type. Analyzing the energies of wild type and mutated podocin structures indicated that the mutated forms were thermodynamically more stable than the wild type, suggesting biological processes favour the synthesis of mutated podocin forms over the wild type. In conclusion, two mutations, G92C (−8179.272 kJ/mol) and P118L (−8136.685 kJ/mol), are more stable and likely to occur in podocin structure compared to the wild type (−8105.622 kJ/mol). Though the differences in energy between mutated and wild type are relatively small (approximately 74 and 35 kJ/mol), these differences may play a crucial role in determining the genetic preference for these mutations.

In the article by Sahu et al., 2020, examining the structural and functional binding performance of proteins related to the genetic basis of diseases is a significant challenge. Podocin, a member of the flotillin, stomatin, and prohibitin family, is expressed in nephron tissue and is involved in type 2 nephrotic syndrome (NPHS2) due to mutations. In this study, we assessed the binding performance of both the native and mutant forms of podocin protein with various phytochemicals such as aegeline and berberine.

According to Sahu et al., 2019, podocin, a protein found in nephrotic tissues, has mutations within its gene that have been linked to familial idiopathic type 2 nephrotic syndrome. In this study, we examined the mutation position R138Q in human podocin protein, located in the SPFH_PODOCIN domain. Molecular docking using AutoDock Vina was conducted to explore the molecular interaction between both mutant and wild-type models of podocin and biologically active compounds from *Boerhaviadiffusa* plant extracts. Boeravinone B and F demonstrated higher binding energy in the mutant protein

compared to other compounds. Additionally, molecular dynamics simulations were performed with a 50 ns trajectory for both the wild-type and mutant models, revealing that flexibility decreases in the mutant protein. The identification of disease-related non-synonymous single nucleotide polymorphisms and the analysis of their impact using computational methods can provide valuable insights for the diagnosis and treatment of conditions such as nephrotic syndrome.

CHAPTER 3

AIM AND SCOPE

3.1. AIM OF THE STUDY

There are millions of people who are suffering from nephrotic syndrome and are undergoing steroid therapy to deal with the consequences of the disease. The aim of this project is to study and analyse the effect of the phytochemicals present in the medicinal plants *Boerhaviadiffusa*, *Paederiafoetida* and *Kalanchoe pinnata* on the disease nephrotic syndrome. This study aims to identify the possible and probable targets for nephrotic syndrome through computer-aided drug discovery processes.

3.2. SCOPE OF THE STUDY

Target detection and identification: With the help of this study, the potential targets for the disease can be identified. The identification of potential targets of nephrotic syndrome can facilitate the treatment and enhance the quality diagnosis of the disease. Identification of the targets can also create scopes for the discovery of new and efficient medication with less side effects for the treatment of nephrotic syndrome.

Enhanced treatment and therapy quality: With the help of this study, the treatment and therapy of the disease may be improved with the help of the probable drug discovered through the study. As nephrotic syndrome's first line of therapy is corticosteroid therapy, the probable discovered drugs might prove to be helpful in the treatment of the disease with lesser side effects.

METHOD AND METHODOLOGY

4.1. COLLECTION OF DATA AND INFORMATION

With the help of Sahu et al., 2019 study the information about the genes is collected from the Gene Entrez of the NCBI (National Library of Medicine) and UniprotKB databases. The information about the probable mutations is collected from the NCBI dbSNP databases. The information about the target protein is obtained from the UniprotKB database. The amino acid sequence of the proteins nephrin and podocin respectively were retrieved from the UniprotKB database having uniprot ID O60500 and Q9NP85.

4.2. IDENTIFICATION OF MUTATIONS AND VARIANTS

The mutation and variant information of the protein targets were retrieved according to Sahu et al., 2019, Sahu et al., 2020 and Sahu et al., 2022 study and were studied from the Expasy database. The studied mutations were present in the Ig4-like domain (122 to 344 amino acid) of the nephrin protein and SPFH_Domain (340 to 343 amino acid) of the podocin protein respectively. The variants bearing the variant ID VAR_13039 for mutation P368S, VAR_13040 for mutation L376V were present in the nephrin protein which were responsible for causing congenital nephrotic syndrome of the Finnish type and the variants bearing the variant ID VAR_010233 for mutation R138Q, VAR_01234 for mutation D160G and VAR_010235 for mutation V180M were responsible for causing the steroid resistant nephrotic syndrome.

4.3. THREE-DIMENSIONAL STRUCTURE GENERATION AND QUALITY CHECK

The three-dimensional structure of the wild and mutant structure was generated with the help of the Robetta server. The mutations were introduced in the amino acid sequence of the target protein manually and the structure of the mutant models were generated separately and individually. The generated structures

were then analysed for their structural quality with the help of PROCHECK and MolProbity websites and the structures with satisfied thresholds were considered for further analysis.

4.4. LIGAND IDENTIFICATION AND STRUCTURE RETRIEVAL.

The phytochemical compounds and their three-dimensional structures were retrieved from the IMPPAT 2.0 database for each of the selected medicinal plants. The SMILES of these structures were collected from IMPPAT 2.0 and saved in excel sheets, which were then used for their filtration on the basis of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties. The SwissADME and pkCSM online websites were used to filter these phytochemicals. The small-molecule tool of Discovery Studio was used to calculate the ADMET descriptors and predict the toxicity of the phytochemicals. The phytochemicals which passed all the thresholds and parameters were considered further for the docking study.

4.5. MOLECULAR DOCKING

The three-dimensional structures of the filtered phytochemical compounds were then docked with the receptor structures using the docking optimization tools, CDOCKER and LigandFit of Discovery Studio. The docking was performed using CDOCKER by applying CHARMM forcefield and full potential final minimization. The simulated annealing was done by using 2000 heating steps with the heat target temperature of 700K and 5000 cooling steps with the cool target temperature of 300K. LigandFit performed docking with the default parameters of the Discovery Studio which produces the maximum accurate results.

These structures were then visualised using the Biovia Discovery Studio. The molecular docking of these structures was also carried out by Discovery Studio. The structures were checked for any unwanted molecules which were then removed and the total energy of the structures were calculated by the simulation tool of the Discovery Studio. After the calculation of the energy, the domain region of the models was determined manually with the use of the

Expasy database and the binding sites present in the domain site were edited and defined.

4.6. *MOLECULAR SIMULATION*

The best docked complexes were subjected to simulation and selected on the basis of their cdocker energy, dock score and the presence of hydrogen bonds. These docked complexes were then run with simulation tool on Discovery studio with standard dynamics cascade and solvation to determine the compatibility of the receptor and ligand molecules.

RESULT AND DISCUSSION

5.1. COLLECTION OF DATA AND INFORMATION

The information about the target protein, nephrin and podocin were collected from the Gene Entrez, NCBI (National Library of Medicine) and UniprotKB databases which revealed the required amino acid sequence of the respective target proteins along with the genetic basis of the target proteins. It also revealed the information about the function and role of the genes NPHS1 and NPSH2 in the body. Both the genes are responsible for coding proteins which are important to maintain the glomerular filtration barrier in the kidney. The NCBI dbSNP database revealed the information about the mutations which are probable for the onset of Nephrotic syndrome and may give rise to the complications in the glomerular filtration barrier.

Table 5.1: Genes associated with Nephrotic Syndrome and their respective information.

Sl. No.	Gene Symbol	Gene Name	Protein	Mutations
1.	NPHS1	Nephrotic Syndrome 1	Nephrin	L376V P368S
2.	NPHS2	Nephrotic Syndrome 2	Podocin	R138Q D160G V180M

5.2. IDENTIFICATION OF MUTATIONS AND VARIANTS

There exists a number of mutations which are responsible for the onset of nephrotic syndromes of different nature. According to the OMIM (Online

Mendelian Inheritance in Man) database, the nephrin protein and podocin protein related mutations were found to be greatly related with nephrotic syndromes. As there exists a high number of mutations related to the NPHS1 and NPHS2 which may cause nephrotic syndrome, literature and research articles were referred for further information. According to literature review, the Ig4-like domain (122 to 344 amino acid) of the nephrin protein and SPFH_Domain (340 to 343 amino acid) of the podocin protein respectively were revealed to be probably prone to mutations, therefore, the mutations P368S and L376V in nephrin protein were considered for the analysis in this study similarly, the mutations R138Q, D160G and V180M were considered for the study analysis.

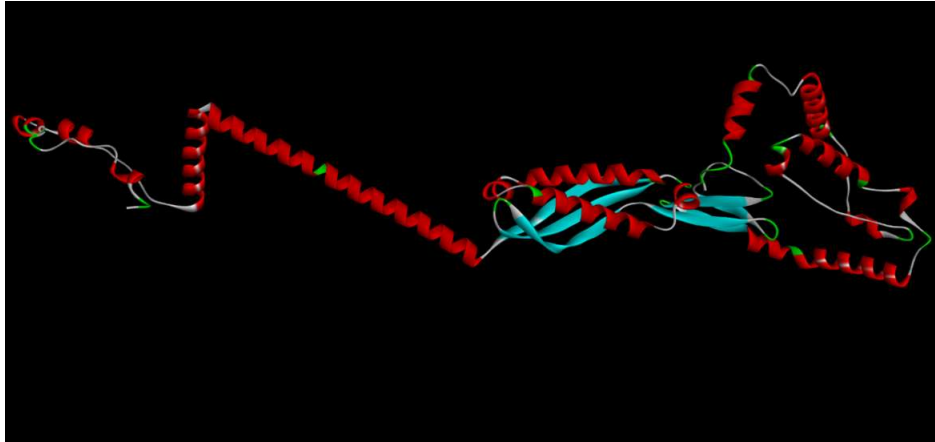
5.3. EFFECT OF MUTATIONS ON THE STRUCTURAL STABILITY OF THE NATIVE MODELS

The stability of the protein structures, nephrin and podocin respectively were analyzed by the SIFT online tool with the use of their amino acid sequence. The substitution at position 368 from P to S of the nephrin protein is predicted to affect the function of the protein with a score of 0.00 and the substitution at position 376 from L to V is predicted to be tolerated with a score of 0.23. Similarly, for the podocin protein the substitution at position 138 from R to Q is predicted to be tolerated with a score of 0.06, the substitution at position 160 from D to G is predicted to affect protein function with a score of 0.00 and the substitution at position 180 from V to M is predicted to be tolerated with a score of 0.09.

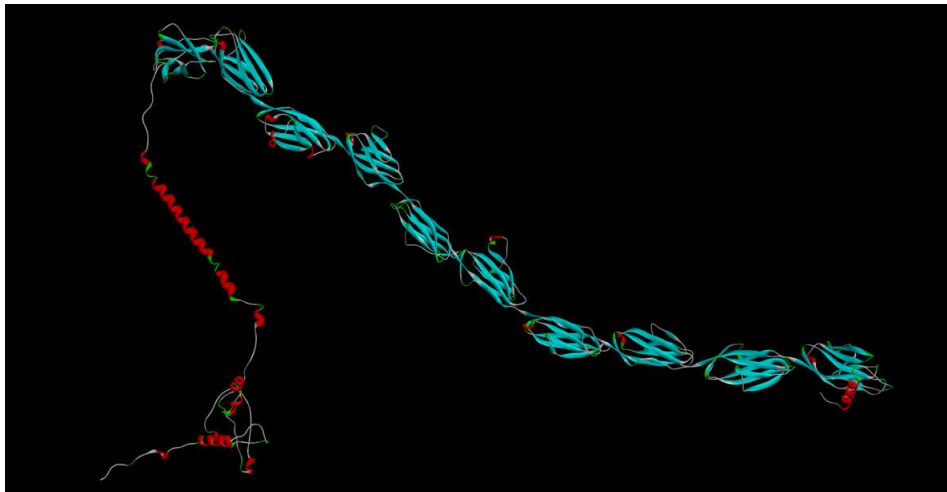
5.4. THREE-DIMENSIONAL STRUCTURE GENERATION

As the existing models of the target proteins did not have pre-existing experimental structures in the Protein Data Bank (PDB) database for the entire amino acid sequence of the proteins and the quality of the alpha fold structures did not pass the threshold of the possible phi/psi angles for an amino acid, the three-dimensional structure of the protein models was in need to be generated. The structures of the protein models were generated with the help of Robetta protein prediction server which uses homology modelling and ab-initio methods

in a hybrid approach to predict protein structures. The features of the Robetta server includes relatively fast and accurate deep learning-based methods like the RoseTTAfold and TrRosetta. It also provides an interactive submission interface which allows custom sequence alignment for homology modelling, constraints, local fragments and many more.



a) Wild model of podocin protein.



b) Wild model of nephrin protein

Fig:5.1: Three-dimensional structure of wild models of the target proteins podocin and nephrin respectively.

5.5. STRUCTURAL QUALITY CHECK

Each of the models generated by the Robetta server is validated by Procheck and MolProbity servers, the models with the highest comparative score and the ones which satisfied the range of parameters were selected. These parameters include MolProbity score, clash score, Ramachandran favourable, etc. All the wild models and the mutant models have a clash score of 99 percentile, Ramachandran favourable of more than 95 percentile and under 99 percentile and a clash score of more than 96 percentile. The results obtained from the MolProbity server for the quality analysis of the wild as well as mutant models of nephrin and podocin protein are given in the following Fig.:5.2.

All-Atom Contacts	Clashscore, all atoms:	1.59	99 th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	1	0.10% Goal: <0.3%
	Favored rotamers	1032	99.42% Goal: >98%
	Ramachandran outliers	11	0.89% Goal: <0.05%
	Ramachandran favored	1190	96.05% Goal: >98%
	Rama distribution Z-score	0.74 ± 0.24	Goal: abs(Z score) < 2
	MolProbity score [†]	1.18	99 th percentile* (N=27675, 0Å - 99Å)
	CP deviations >0.25Å	0	0.00% Goal: 0
	Bad bonds:	10 / 9724	0.10% Goal: 0%
	Bad angles:	26 / 13284	0.20% Goal: <0.1%
Peptide Omegas	Cis Prolines:	10 / 99	10.10% Expected: ≤1 per chain, or ≤5%
	Cis nonProlines:	5 / 1141	0.44% Goal: <0.05%
	Twisted Peptides:	1 / 1240	0.08% Goal: 0
Low-resolution Criteria	CaBLAM outliers	42	3.4% Goal: <1.0%
	CA Geometry outliers	14	1.13% Goal: <0.5%
Additional validations	Chiral volume outliers	0/1498	
	Waters with clashes	0/0	0.00% See UnDowser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.
* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.
[†] MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.
Key to table colors and cutoffs here: [?](#)

(a)

All-Atom Contacts	Clashscore, all atoms:	2.18	99 th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	0	0.00% Goal: <0.3%
	Favored rotamers	1034	99.61% Goal: >98%
	Ramachandran outliers	10	0.81% Goal: <0.05%
	Ramachandran favored	1193	96.29% Goal: >98%
	Rama distribution Z-score	1.48 ± 0.23	Goal: abs(Z score) < 2
	MolProbity score [†]	1.24	99 th percentile* (N=27675, 0Å - 99Å)
	CP deviations >0.25Å	0	0.00% Goal: 0
	Bad bonds:	9 / 9721	0.09% Goal: 0%
	Bad angles:	24 / 13279	0.18% Goal: <0.1%
Peptide Omegas	Cis Prolines:	10 / 98	10.20% Expected: ≤1 per chain, or ≤5%
	Cis nonProlines:	5 / 1142	0.44% Goal: <0.05%
	Twisted Peptides:	1 / 1240	0.08% Goal: 0
Low-resolution Criteria	CaBLAM outliers	45	3.6% Goal: <1.0%
	CA Geometry outliers	11	0.89% Goal: <0.5%
Additional validations	Chiral volume outliers	0/1498	
	Waters with clashes	0/0	0.00% See UnDowser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.
* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.
[†] MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.
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(b)

All-Atom Contacts	Clashscore, all atoms:	1.81	99 th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	0	0.00% Goal: <0.3%
	Favored rotamers	1032	99.42% Goal: >98%
	Ramachandran outliers	8	0.65% Goal: <0.05%
	Ramachandran favored	1185	95.64% Goal: >98%
	Rama distribution Z-score	0.71 ± 0.24	Goal: abs(Z score) < 2
	MolProbity score [^]	1.24	99 th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	0	0.00% Goal: 0
	Bad bonds:	5 / 9722	0.05% Goal: 0%
	Bad angles:	25 / 13280	0.19% Goal: <0.1%
Peptide Omegas	Cis Prolines:	9 / 98	9.18% Expected: ≤1 per chain, or ≤5%
	Cis nonProlines:	2 / 1142	0.18% Goal: <0.05%
Low-resolution Criteria	CaBLAM outliers	43	3.5% Goal: <1.0%
	CA Geometry outliers	6	0.49% Goal: <0.5%
Additional validations	Chiral volume outliers	0/1498	
	Waters with clashes	0/0	0.00% See UnDowser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.

* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.

[^] MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.

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(c)

All-Atom Contacts	Clashscore, all atoms:	1.83	99 th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	0	0.00% Goal: <0.3%
	Favored rotamers	322	99.38% Goal: >98%
	Ramachandran outliers	2	0.52% Goal: <0.05%
	Ramachandran favored	373	97.90% Goal: >98%
	Rama distribution Z-score	1.37 ± 0.42	Goal: abs(Z score) < 2
	MolProbity score [^]	0.97	100 th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	0	0.00% Goal: 0
	Bad bonds:	0 / 3019	0.00% Goal: 0%
	Bad angles:	6 / 4080	0.15% Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 25	0.00% Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	6	1.6% Goal: <1.0%
	CA Geometry outliers	6	1.58% Goal: <0.5%
Additional validations	Chiral volume outliers	0/465	
	Waters with clashes	0/0	0.00% See UnDowser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.

* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.

[^] MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.

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(d)

All-Atom Contacts	Clashscore, all atoms:	1.33	99 th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	0	0.00% Goal: <0.3%
	Favored rotamers	323	99.69% Goal: >98%
	Ramachandran outliers	2	0.52% Goal: <0.05%
	Ramachandran favored	368	96.59% Goal: >98%
	Rama distribution Z-score	1.51 ± 0.44	Goal: abs(Z score) < 2
	MolProbity score [^]	1.08	100 th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	0	0.00% Goal: 0
	Bad bonds:	0 / 3016	0.00% Goal: 0%
	Bad angles:	3 / 4074	0.07% Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 24	0.00% Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	12	3.2% Goal: <1.0%
	CA Geometry outliers	3	0.79% Goal: <0.5%
Additional validations	Chiral volume outliers	0/465	
	Waters with clashes	0/0	0.00% See UnDowser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.

* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.

[^] MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.

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(e)

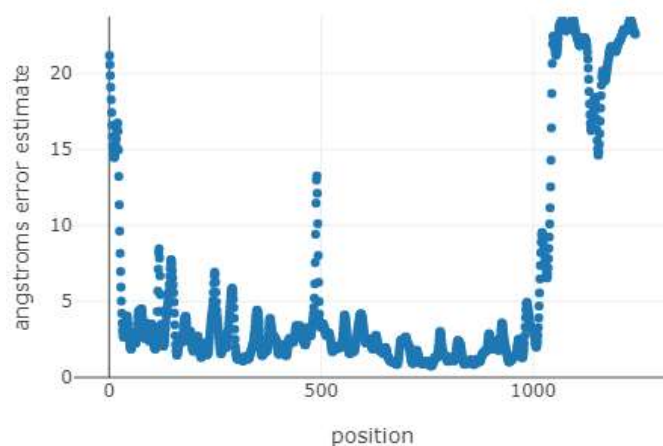
All-Atom Contacts	Clashscore, all atoms:	2.33	99 th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	0	0.00% Goal: <0.3%
	Favored rotamers	321	99.38% Goal: >98%
	Ramachandran outliers	1	0.26% Goal: <0.05%
	Ramachandran favored	373	97.90% Goal: >98%
	Rama distribution Z-score	1.55 ± 0.44	Goal: abs(Z score) < 2
	MolProbity score [†]	1.04	100 th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	0	0.00% Goal: 0
	Bad bonds:	0 / 3015	0.00% Goal: 0%
Peptide Omegas	Bad angles:	9 / 4074	0.22% Goal: <0.1%
	Cis Prolines:	0 / 25	0.00% Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	11	2.9% Goal: <1.0%
	CA Geometry outliers	5	1.32% Goal: <0.5%
Additional validations	Chiral volume outliers	0/464	
	Waters with clashes	0/0	0.00% See UnDownser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.
* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.
[†] MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.
Key to table colors and cutoffs here: [?](#)

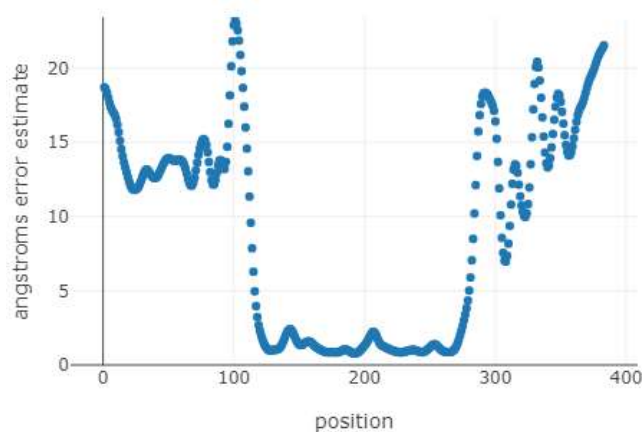
(f)

Fig.:5.2: Quality analysis parameters of the generated three-dimensional target protein structures such as clash score, Ramachandran favoured and molprobity scores of: (a) Nephrin full structure, (b) Nephrin all mutations, (c) Nephrin P368S mutant model, (d) Podocin full structure, (e) Podocin all mutations, (f) Podocin D160G mutant model.

Similarly, the wild models were also considered on the basis of their angstrom error estimate vs position plot generated by the robetta server, where lesser angstroms error estimate means greater greater quality of the structure. The armstrong error estimate graph for the wild models of nephrin and podocin protein are depicted in fig.A and fig.B respectively.



A.



B.

Fig:5.3: A. Armstrong error estimate vs position graph for nephrin and B. Armstrong error estimate vs position graph for Podocin.

5.6. PHYTOCHEMICAL SCREENING

The SMILES of the plant compounds *Boerhaviadiffusa*, *Paederiafoetida* and *Kalanchoe pinnata* were retrieved from the IMPPAT 2.0 database and were screened in the tools, namely SwissADME and pkCSM. The three-dimensional structures of the filtered phytochemicals were subjected to filtration once again in Discovery studio for their physicochemical properties (ADME-Tox). The phytochemicals which satisfied the threshold values and TOPKAT predictions

were considered for further evaluation and study. The compounds which passed the filtration and screening parameters are listed in table.1.

Table 5.1: ADME-Toxicity screened phytochemicals.

Paederia foetida							
Compound name	ADMET_CYP2D6	Hepatotoxicity	Rat Oral LD50	Skin Irritant	Skin Sensitiza	Biodegradability	TOPKAT_Mouse_Carcinogen
Deacetylasperuloside	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Scandoside	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Geniposide	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Bryophyllumpinnatum							
1,3,3-Trimethyl-2-oxabicyclo[1.1.0]	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
1-Octen-3-OL	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen

6-Epi-beta-bisabolol	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Bersaldegennin 3-acetate	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Bryophyllin A	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Bryophyllin B	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Caffeic acid	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Cedrelanol	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Eucalypto	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Isocitric acid	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen

Pulegone	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Viridiflorol	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Boerhaviadiiffusa							
Boeravinone C	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Boeravinone D	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Boeravinone E	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Boerhadiffusene	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen
Punarnavoside	False	False	False	Non-irritant	Weak	Degradable	Non-carcinogen

5.7. MOLECULAR DOCKING

The three-dimensional of the filtered ligand compounds were compiled in individual files for all the three medicinal plants. The generated three-dimensional structures of the target proteins were prepared for docking. Missing hydrogen bonds were added to the target proteins to improve the quality of the proteins and the energy of the protein structures were calculated with the help of Biovia Discovery studio client 2021 software.

Table 5.2: The wild and mutant models of the proteins and their respective calculated energy and minimized energies.

Protein Name	Type	Calculated Energy	Minimized energy
Nephrin	Wild	-41635 kcal/mol	-66942 kcal/mol
Nephrin	Mutant	-41177 kcal/mol	-67953 kcal/mol
Nephrin_P368S	Mutant	-41299 kcal/mol	-67377 kcal/mol
Nephrin_L376V	Mutant	-41088 kcal/mol	-66999 kcal/mol
Podocin	Wild	-12395 kcal/mol	-19950 kcal/mol
Podocin	Mutant	-12842 kcal/mol	-19755 kcal/mol
Podocin_R138Q	Mutant	-12278 kcal/mol	-19718 kcal/mol
Podocin_D160G	Mutant	-12097 kcal/mol	-19710 kcal/mol
Podocin_V180M	Mutant	-12549 kcal/mol	-19344 kcal/mol

All the models were successfully minimised, however the quality of most of the models deteriorated after the minimization and their residues got scattered in the disallowed region of the Ramachandran graph. But there was no change

observed in the minimised structures of the Podocin wild model, Podocin_R138Q model and Podocin_V180M model and their minimised structures were considered for the docking studies. The three-dimensional structures of the target proteins were edited and the binding sites were defined from their receptor cavities. The domain regions of the proteins were determined as these regions are important for their function in the context of the kidney's filtration barrier. The binding sites present in the domain region were selected for the docking purpose.

The files containing all the filtered three-dimensional structures of the phytochemicals were then docked with each defined site of the target protein by cdocker and LiganFit docking methods. CHARMM force field was applied and each of the interactions were visualized. The docked complex with the highest dock score and best pose were selected from each of the three medicinal plants. For further analysis, the best interactions from the three plants were compared and the best among these three interactions was considered for simulation.

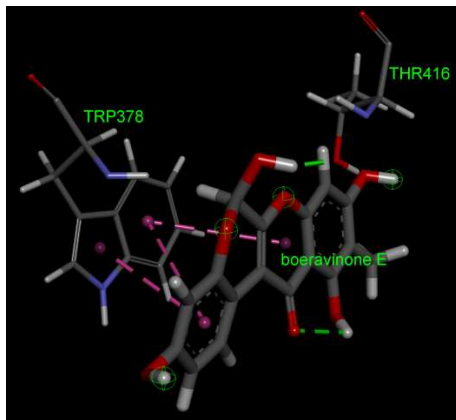
The phytochemical ligand molecules that showed the best interaction included eucalyptol from the plant *Kalanchoe pinnata*, boerhavinone C from the plant *Boerhaviadiffusa*, and caffeic acid from the plant *Kalanchoe pinnata* with the different models of podocin protein. Similarly, the phytochemical compounds that depicted good interaction with the different models of nephrin models included boerhavinone C from the plant *Boerhaviadiffusa* and isocitric acid from the plant *Kalanchoe pinnata*.

Table 5.3: Molecular docking results.

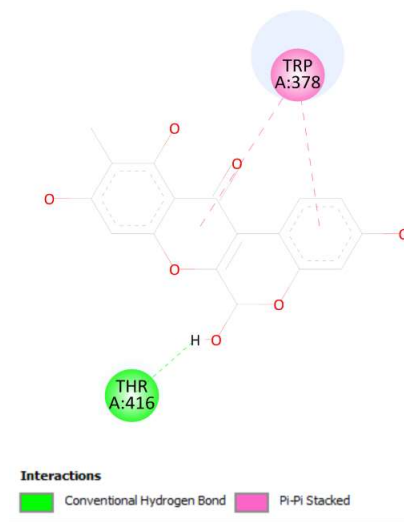
Target Protein	Target Nature	Plant Name	Compound Name	Dock Score
Nephrin	Wild	<i>Boerhavia diffusa</i>	Boerhavinone E	28.87
Nephrin	Mutant	<i>Kalanchoe pinnata</i>	Isocitric acid	28.41

Nephrin	Mutant_P368 S	<i>Kalanchoe pinnata</i>	Isocitric acid	25.85
Nephrin	Mutant_L376V	<i>Kalanchoe pinnata</i>	Caffeic acid	20.47
Podocin	Wild	<i>Kalanchoe pinnata</i>	Eucalyptol	10.27
Podocin	Mutant	<i>Boerhaviadiffusa</i>	BoerhavinoneC	34.39
Podocin	Mutant_R138 Q	<i>Kalanchoe pinnata</i>	Caffeic acid	17.17
Podocin	Mutant_D160 G	<i>Boerhaviadiffusa</i>	BoerhavinoneC	34.29
Podocin	Mutant_V180 M	<i>Kalanchoe pinnata</i>	Isocitric acid	26.49

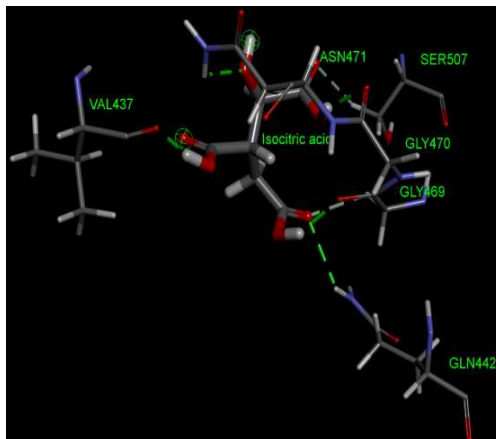
From the above analysis, the docked complexes with the highest scores for both the targets were considered for the simulation study. From the above analysis, it can be observed that the phytochemicals boerhavinone E, boerhavinone C and isocitric acid showed best interactions with the target proteins and therefore, were considered further and are shown below.



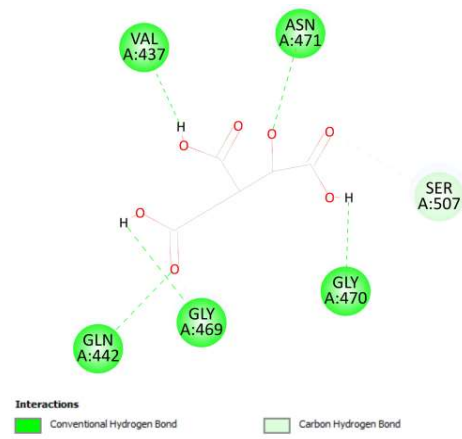
(a)



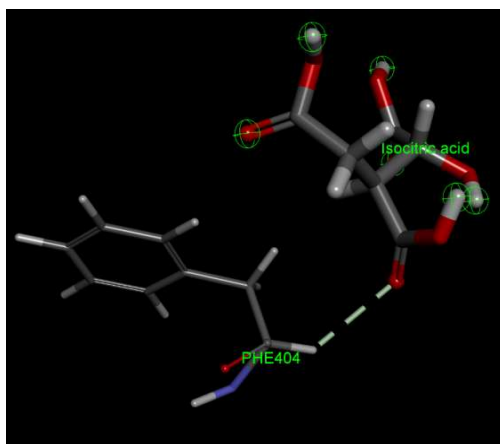
(b)



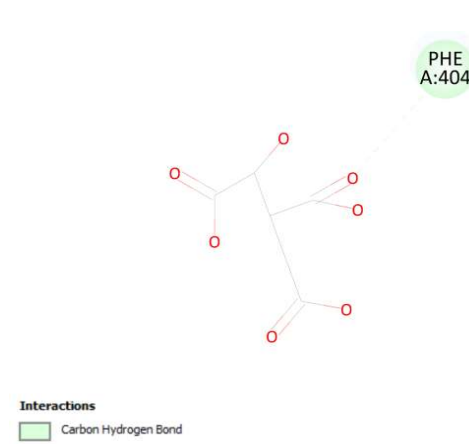
(c)



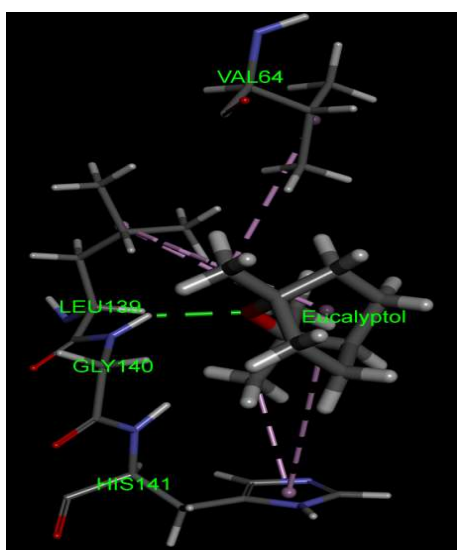
(d)



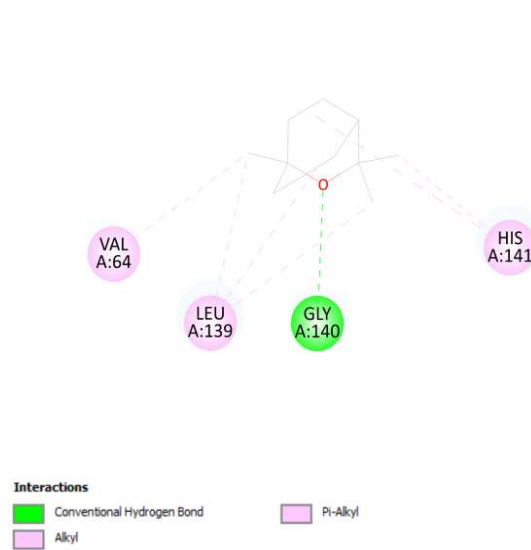
(e)



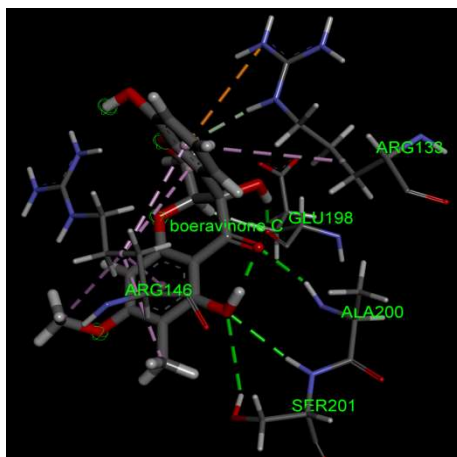
(f)



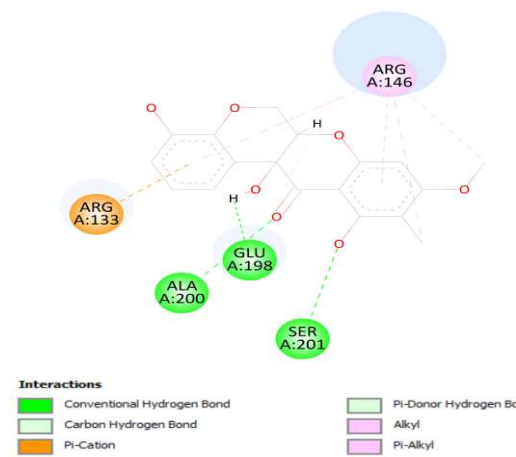
(g)



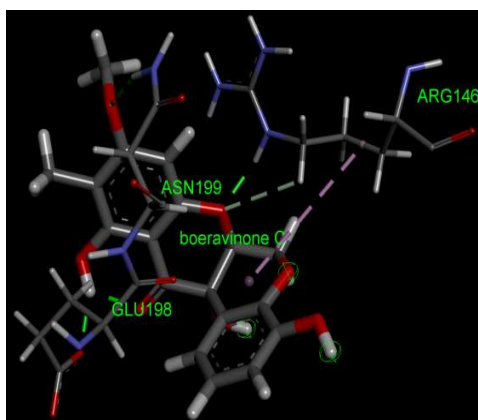
(h)



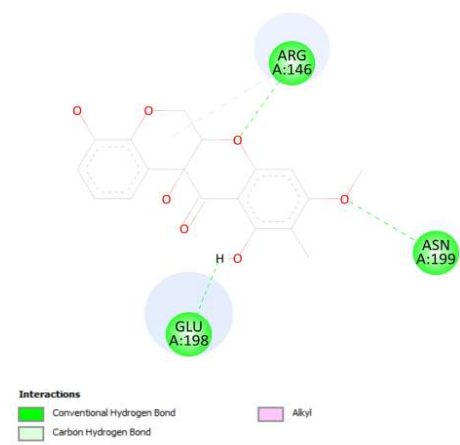
(i)



(j)



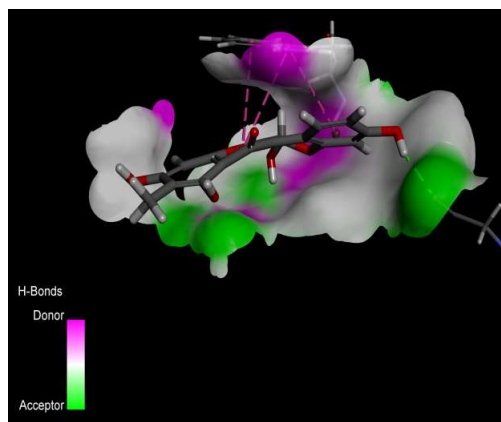
(k)



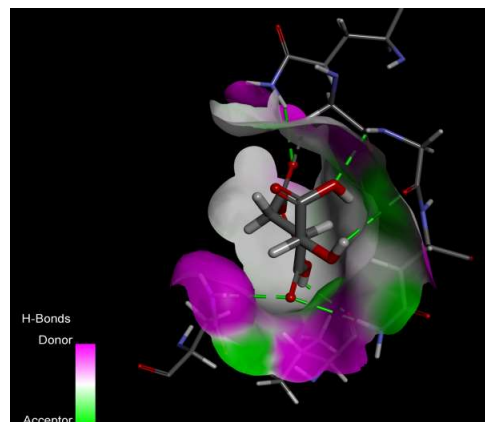
(l)

Fig:5.4: The best interactions between the target proteins and the phytochemical compounds: (a) 3D interaction of Nephrin wild model-boerhavinone E ; (b) 2D interaction of Nephrin wild model-boerhavinone E; (c) 3D interaction of Nephrin mutant model-Isocitric acid; (d) 2D interaction of Nephrin mutant model-Isocitric acid; (e) 3D interaction of Nephrin mutant_P368S model-Isocitric acid; (f) 2D interaction of Nephrin mutant_P368S model-Isocitric acid; (g) 3D interaction of Podocin wild model-eucalyptol; (h) 2D interaction of Podocin wild model-eucalyptol; (i)

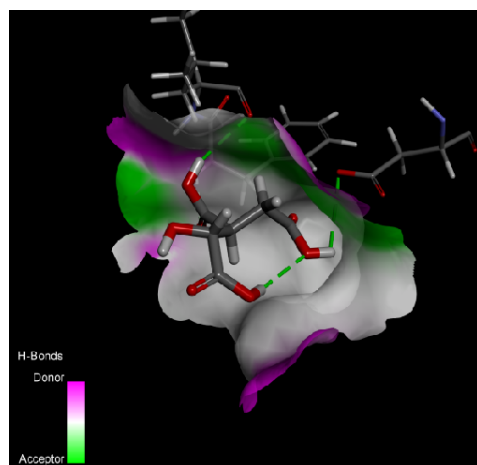
3D interaction of Podocin mutant model-boerhavinone C; (j) 2D interaction of Podocin mutant model-boerhavinone C; (k) 3D interaction of Podocin mutant_D160G-boerhavinone C.



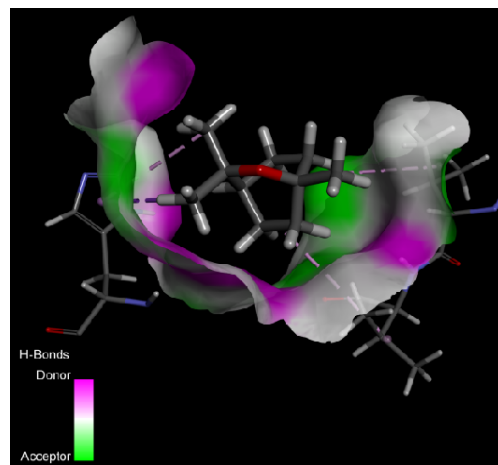
(a)



(b)



(c)



(d)

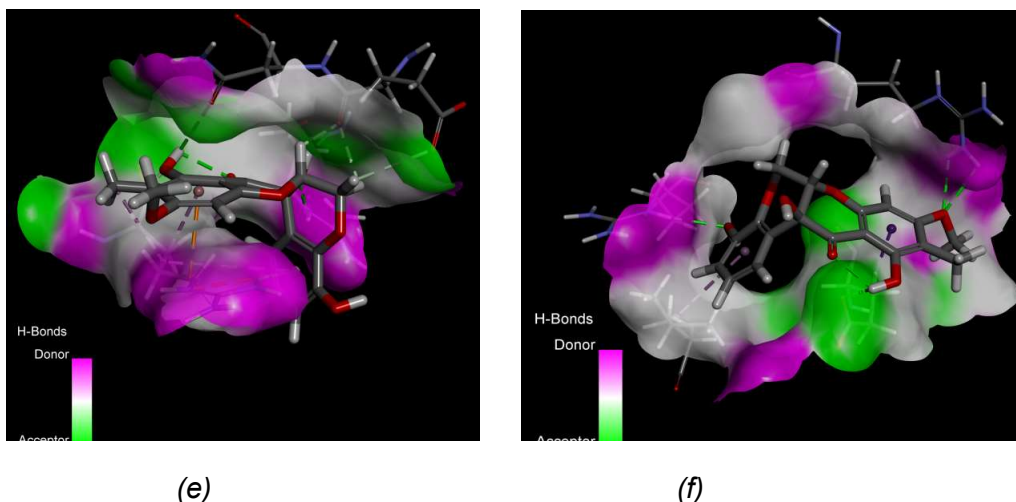


Fig:5.5: Hydrogen bond donor and acceptor residues in the pocket view of: (a) Nephrin wild model-boerhavinone E complex, (b) Nephrin all mutation model-isocitric acid complex, (c) Nephrin mutant_P368S-isocitric acid complex, (d)Podocin wild model- eucalyptol complex, (e) Podocin all mutations-boerhavinone C complex, (f) Podocin mutant D160G-boerhavinone C complex.

5.8. MOLECULAR SIMULATION

Molecular dynamics simulation studies have been performed to observe and analyze the different conformational changes present in the interactions of the wild and mutant type of the target proteins nephrin and podocin respectively. It provides a detailed view of dynamic behaviour of protein-ligand complexes over time. Molecular dynamic simulation explains the binding mechanism of ligands to their protein targets. It provides valuable insights for drug discovery, ligand optimizations, etc.

The best docked complexes with the highest scores were considered for the simulation studies and exposed for further analysis. Each of the complexes was saved in protein data bank (pdb) format and was exposed to standard dynamic cascade simulation processes.

The parameters for the process were set with the first minimization algorithm to be the conjugate gradient in a maximum of 5000 steps with a root mean square (rms) gradient of 1.0. The second minimization algorithm was steepest descent with 5000 maximum steps and the root mean square (rms) gradient of 0.1. The heating parameter was set to be completed in the simulation time of 10ps with the time steps of 4fs. The initial temperature for heating was 50K and the target temperature was set to be 300K. The frequency of adjusting velocity was 50 and the result for heating was saved after every 2ps. The simulation time for equilibrium was set to be 10ps in time steps of 2fs. The target temperature for equilibrium was 300K and the results of equilibrium were set to save after every 2ps. For the production parameter, the simulation time was set to 10ps in the time steps of 2fs. The target temperature was 300K and the temperature for temperature coupling decay time was set to be 5. The type for production parameter was set to the NVT and the file was saved at an interval of 2ps. With these parameters the standard dynamic cascade process was run.

The solvation analysis was further performed on the standard dynamic cascade analyzed complexes. The default parameters for the solvation were considered for the most accurate results. The standard dynamic cascade and solvation analysis for docked complexes is given in the following table.

Table 5.4: The standard dynamic cascade and solvation results.

Target protein	Interacting phytochemical	Total energy	Solvation molecules
Nephrin wild model	Boerhavinone E	-13,760.3 kcal/mol	335169 water, 78 sodium and 84 chlorides
Nephrin mutant model	Isocitric acid	-13,339.1 kcal/mol	198214 water, 558 sodium and 525 chlorides
Nephrin mutant_P368S model	Isocitric acid	-14,034.1 kcal/mol	322742 water, 888 sodium and 855 chlorides

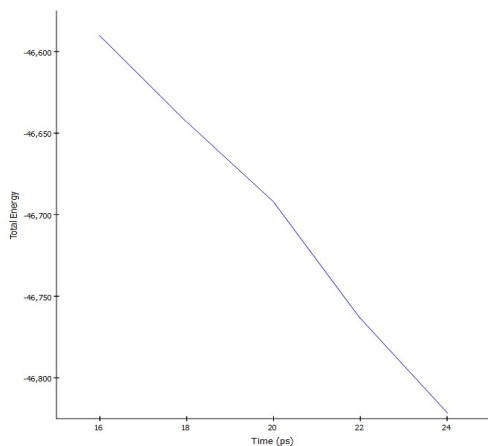
Podocin wild model	Eucalyptol	-46,821.5 kcal/mol	29542 water, 78 sodium and 84 chlorides
Podocin mutant model	BoerhavinoneC	-48,585.4 kcal/mol	35973 water, 95 sodium and 101 chlorides
Podocin mutant_D160G model	BoerhavinoneC	-46,547.4 kcal/mol	34506 water, 91 sodium and 98 chlorides

From the above analysis we can see that the total energy of the docked complexes varies in the mutant forms from the wild forms. The total energy of the nephrin wild model is lesser than the nephrin mutant with all the mutations induced together however, higher than the nephrin mutant model P368S which implies that the nephrin mutant model P368S has a more stable conformation than the wild model of the nephrin protein whereas the nephrin wild mode is more stable when compared to the nephrin mutant with all the mutations induced together. Similarly, in the case of the podocin protein, the total energy for the wild model of podocin protein is greater than the podocin mutant model with the mutations induced together and lesser than the mutant model D160G, which implies that the podocin mutant model with the mutations induced together is the most stable conformation according to the standard dynamic cascade simulation analysis.

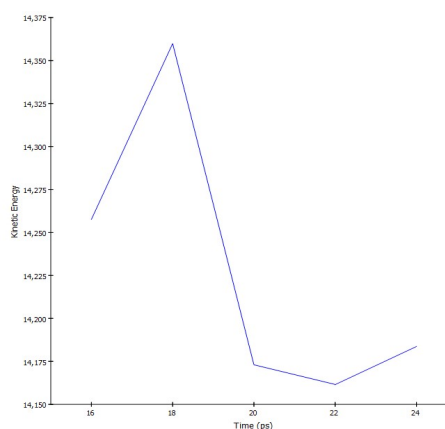
For the solvation analysis, the nephrin wild model contains the highest number of water molecules when compared to the other two mutant models of nephrin and also contain lesser number chloride and sodium molecules which implies that the wild model has the best solubility. In between the two mutant models, the mutant model P368S has more water molecules, sodium and chloride molecules ratio when compared to the nephrin mutant model with all the mutant models induced together hence, the mutant model P368S is more soluble. Similarly, for podocin protein, the podocin wild model has much fewer water molecules and hence has the least solubility. The mutant model of podocin protein with all the mutations induced together has more water molecules when

compared to the podocin mutant model D160G therefore, the podocin mutant model with all the mutations induced together has the second-best solubility.

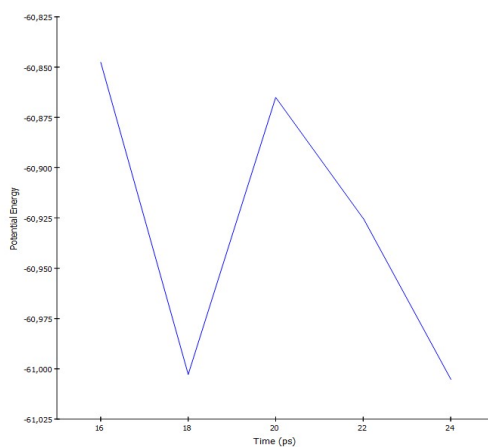
The standard dynamic cascade analysis of the selected docked complexes of both the target proteins nephrin and podocin, revealed the following graphs for their total energy, kinetic energy, potential energy and temperature fluctuations at different time steps of the process. Standard dynamic cascade also helps determine the stability of the complexes at different time steps. The graphs of the total energy, kinetic energy, potential energy and temperature vs time is depicted in Fig:5.6.



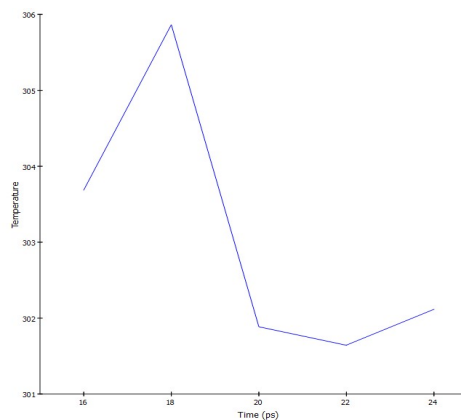
1(a)



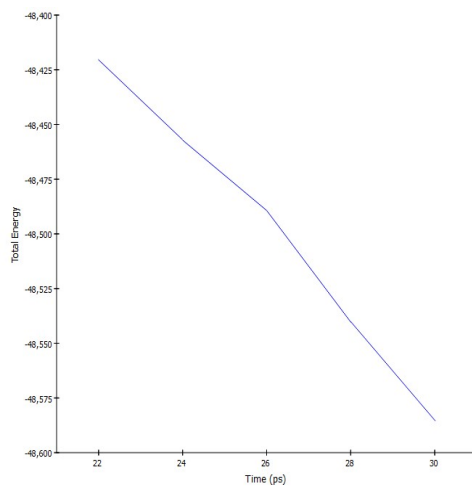
1(b)



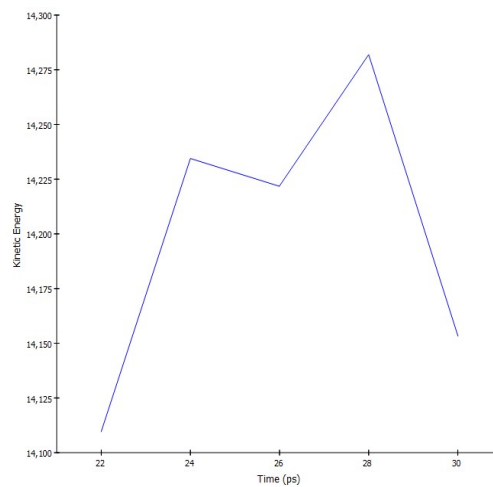
1(c)



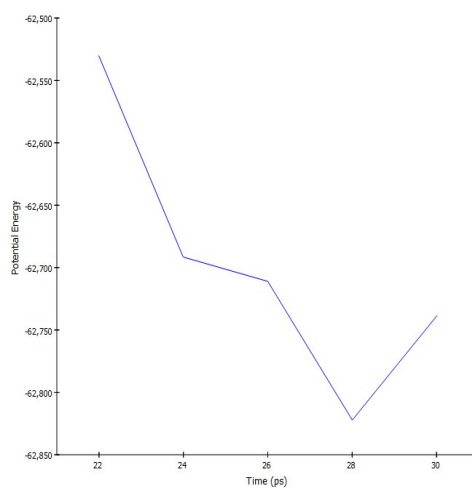
1(d)



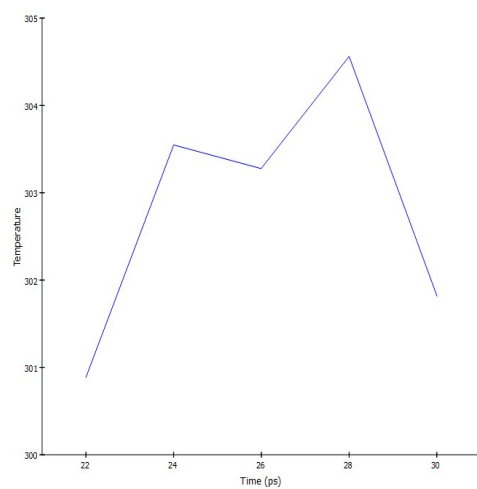
2(a)



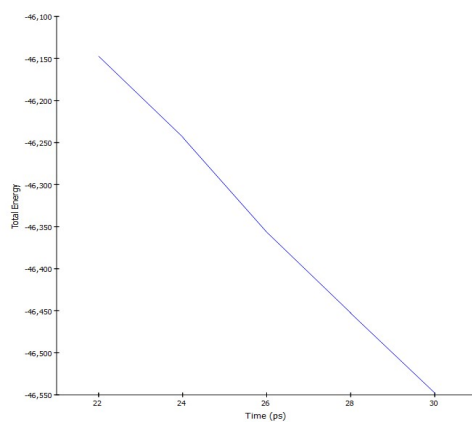
2(b)



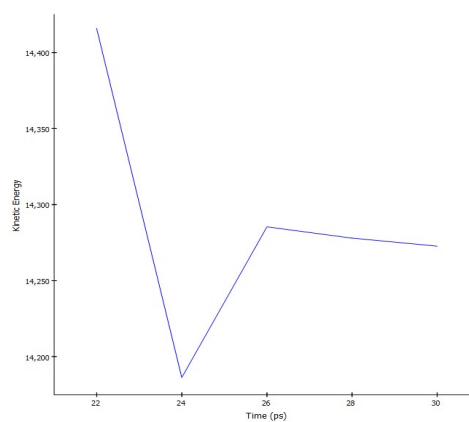
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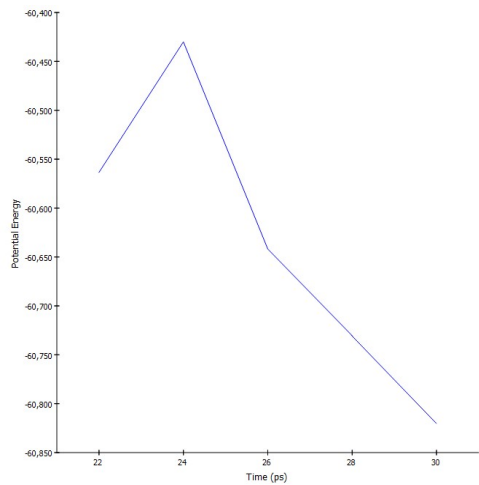
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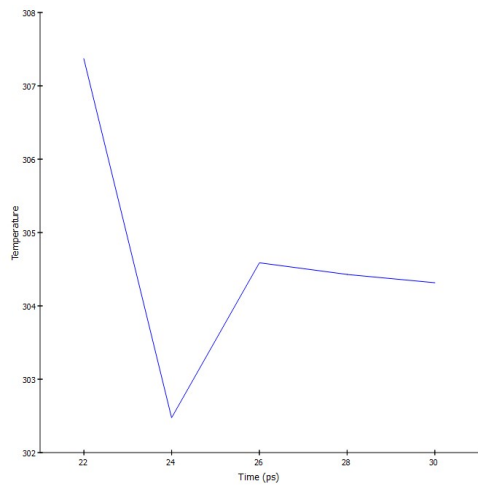
3(a)



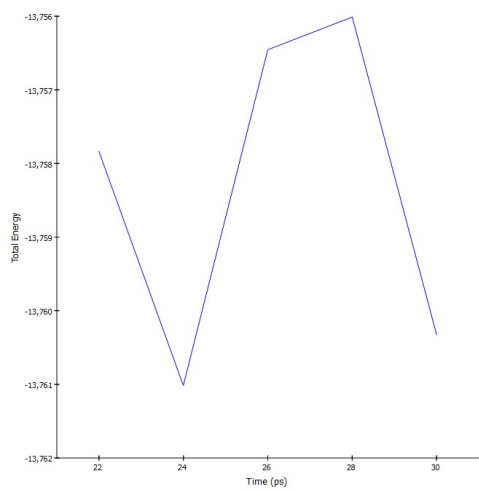
3(b)



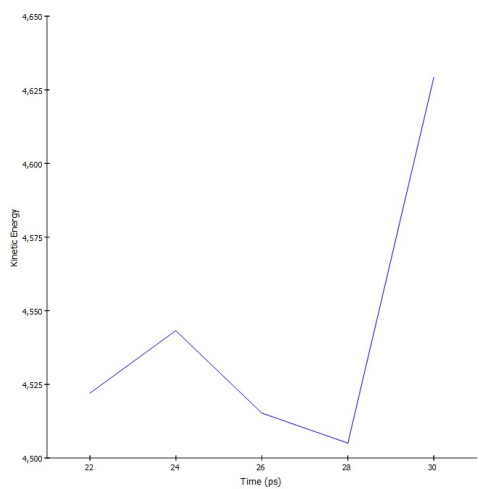
3(c)



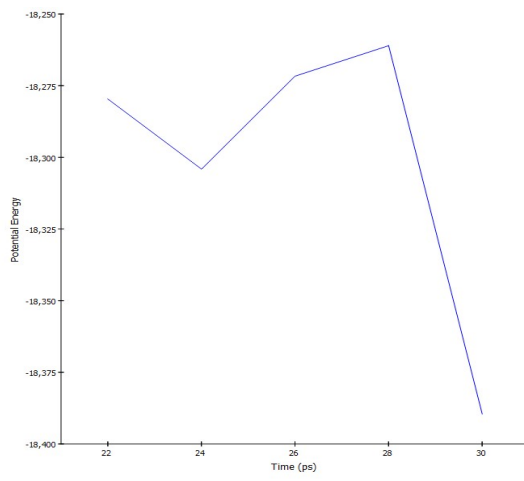
3(d)



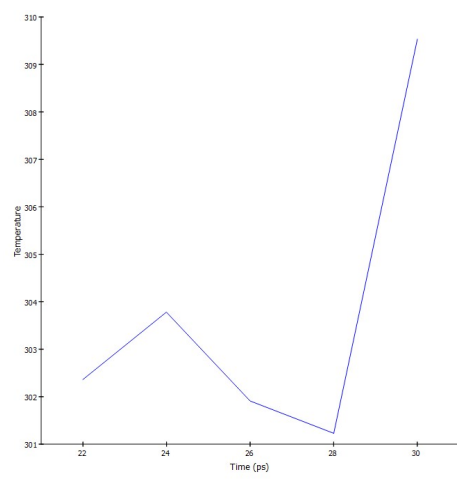
4(a)



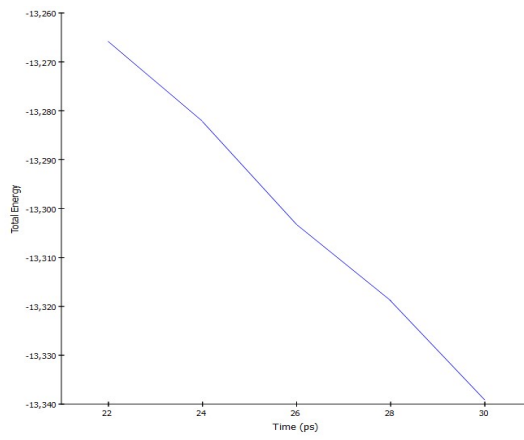
4(b)



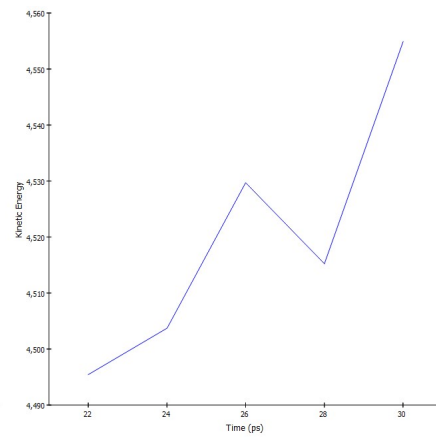
4(c)



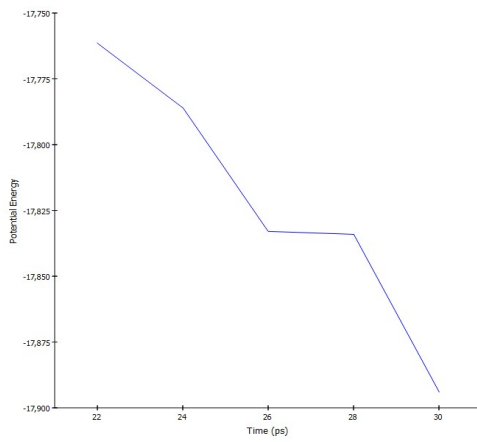
4(d)



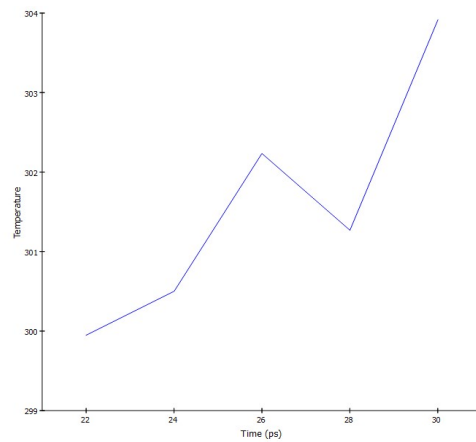
5(a)



5(b)



5(c)



5(d)

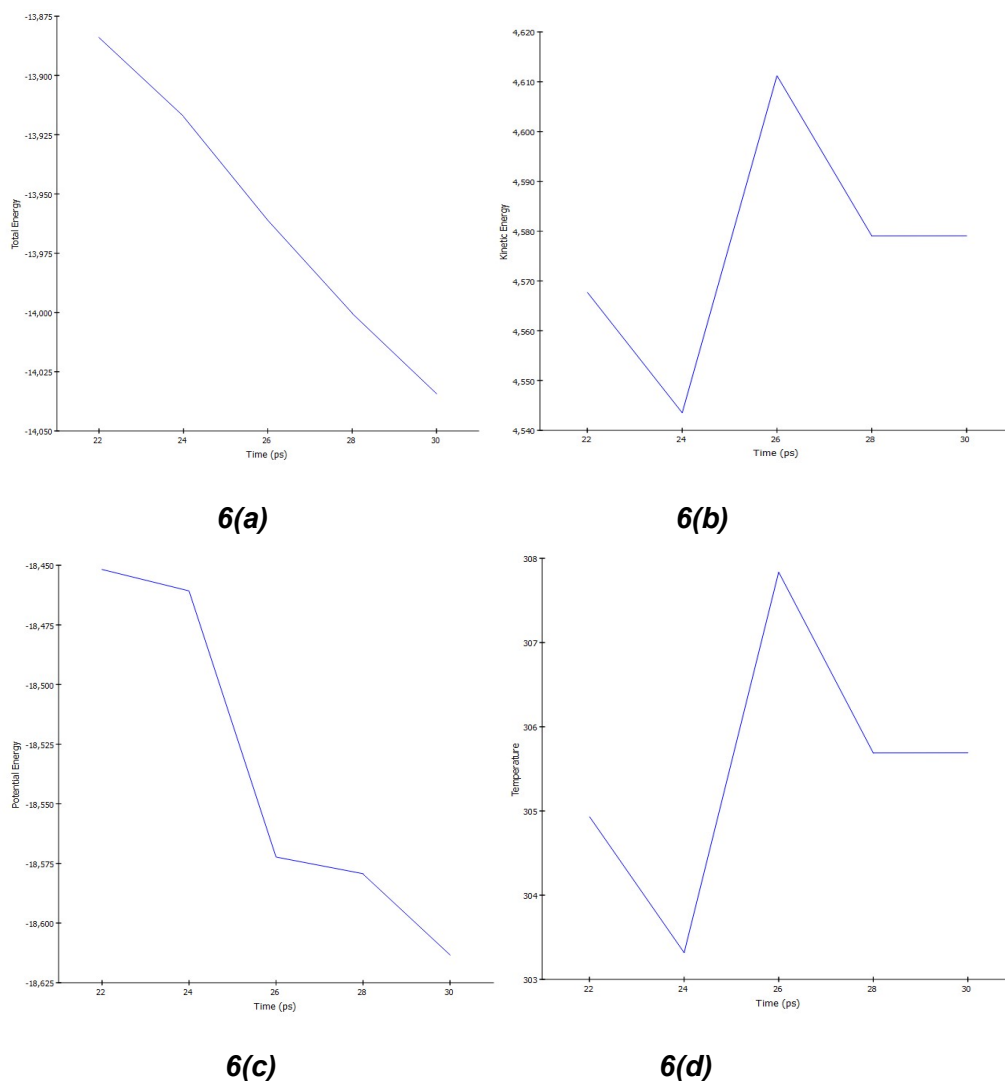


Fig:5.6: Energy vs time graphs for the docked complexes of : 1(a) Total energy vs time graph for nephrin wild model-boerhavinone E complex, 1(b) Kinetic energy vs time graph for nephrin wild model-boerhavinone E complex, 1(c) Potential energy graph vs time graph for nephrin wild model-boerhavinone E complex, 1(d) Temperature vs time graph for nephrin wild model-boerhavinone E complex, 2(a) Total energy vs time graph for nephrin mutant model with all the mutations-isocitric acid complex, 2(b) Kinetic energy vs time graph for nephrin mutant model with all the mutations-isocitric acid complex, 2(c) Potential energy graph vs time graph for nephrin mutant model with all the mutations-isocitric acid complex, 2(d) Temperature vs time graph for mutant model with all the

mutations-isocitric acid complex, 3(a) Total energy vs time graph for nephrin mutant model P368S-isocitric acid complex, 3(b) Kinetic energy vs time graph for nephrin mutant model P368S-isocitric acid complex, 3(c) Potential energy graph vs time graph for nephrin mutant model P368S-isocitric acid complex, 3(d) Temperature vs time graph for mutant model P368S-isocitric acid complex, 4(a) Total energy vs time graph for podocin wild model-eucalyptol complex, 4(b) Kinetic energy vs time graph for podocin wild model-eucalyptol complex, 4(c) Potential energy graph vs time graph for podocin wild model-eucalyptol complex, 4(d) Temperature vs time graph for podocin wild model-eucalyptol complex, 5(a) Total energy vs time graph for podocin mutant model with all the mutations-boerhavinone C complex, 5(b) Kinetic energy vs time graph for podocin mutant model with all the mutations-boerhavinone C complex, 5(c) Potential energy graph vs time graph for podocin mutant model with all the mutations-boerhavinone C complex, 5(d) Temperature vs time graph for podocin mutant model with all the mutations-boerhavinone C complex, 6(a) Total energy vs time graph for podocin mutant model D160G-boerhavinone C complex, 6(b) Kinetic energy vs time graph for podocin mutant model D160G-boerhavinone C complex, 6(c) Potential energy graph vs time graph for podocin mutant model D160G-boerhavinone C complex, 6(d) Temperature vs time graph for podocin mutant podocin mutant model D160G-boerhavinone C complex.

According to the plots obtained from the Discovery studio, the total energy graphs show a fall in the steep for the majority of the complexes, which means that the total energy of the complexes reduced and the complexes attained a more stable configuration. However, for the podocin wild model-eucalyptol complex, the total energy gradient of the complex falls from time 22 ps to 24 ps and again from 28 ps to 30. Therefore, the complex in these periods of time tends to attain the most stable configuration.

For the kinetic energy vs time graph of the complexes, it depicts the changes in the complexes in motion or movement of the complexes with time. For the nephrin wild model-boerhavinone C complex, the kinetic energy of the complex

has the least value from time 20 ps to 22 ps and attains the most stable configuration. For the nephrin all mutations mutant model-isocitric acid complex, the kinetic energy of the complex drops from the time 22 ps to 30 ps and tends to attain a stable configuration. For the nephrin P368S mutant model-isocitric acid complex, the kinetic energy graph for the complex drops from the time 22 ps to 24 ps and gains a stable configuration from time 26 ps to 30 ps. For the podocin wild model-eucalyptol complex, the kinetic energy of the complex is the least from the time 26 ps to 28 ps and tends to attain the stable configuration. For the podocin all mutations mutant model-boerhavinone E complex, the kinetic energy of the complex shows the least value from time 22 ps to 24 ps and hence tends to possess a stable configuration. For the podocin D160G mutant model-boerhavinone E complex, the kinetic energy of the complex shows a downward gradient from time 22 ps to 34 ps and shows a stable configuration from time 28 ps to 30 ps.

For the potential energy of the nephrin wild model-boerhavinone C complex, it rapidly decreases from time 16 ps to 18 ps and from time 20 ps to 24 ps and hence in these durations attains the stable configuration. In molecular docking, low potential energy corresponds to a high binding affinity between the two components of the complex. This high binding affinity is indicative of a strong interaction and, therefore, greater stability. For the nephrin all mutations mutant model-isocitric acid complex, the potential energy graph of the complex shows a downward gradient from time 28 ps to 30 ps and the complex tends to attain the stable configuration during this period of time. For the nephrin P368S mutant model-isocitric acid complex, the potential energy graph of the complex shows a continuous downward gradient from time 24 ps to 30 ps and attains the least value at 30 ps hence, the complex tends to attain a stable configuration at time step of 30 ps. For the podocin wild model-eucalyptol complex, the potential energy graph of the complex shows a downward gradient from time 28 ps to 30 ps and attains the least value at 30 ps hence, the complex tends to attain a stable configuration at time step of 30 ps. For the podocin all mutations mutant model-boerhavinone E complex, the potential energy graph of the complex shows a continuous downward gradient from time

22 ps to 30 ps and attains the least value at 30 ps hence, the complex tends to attain a stable configuration at time step of 30 ps. For the podocin D160G mutant model-boerhavinone E complex also, the potential energy graph of the complex shows a continuous downward gradient from time 22 ps to 30 ps and attains the least value at 30 ps hence, the complex tends to attain a stable configuration at time step of 30 ps.

The temperature vs time graph of the nephrin wild model-boerhavinone C complex shows a steep downward gradient from time 18 ps to 20 ps, and a slanted downward steep from time 20 ps to 22 ps, hence the complex tends to attain the most stable configuration in 18 ps to 22 ps of time. For the nephrin all mutations mutant model-isocitric acid complex, the temperature graph of the complex shows a slanted downwards gradient from time 24 ps to 26 ps and tends to possess the stable configuration in this period of time. For the nephrin P368S mutant model-isocitric acid complex, the temperature graph of the complex shows a steep downward gradient from time 22 ps to 24 ps and tends to attain a stable configuration from time 26 ps to 30 ps. For the podocin wild model-eucalyptol complex, the temperature graph of the complex shows a slanted downwards gradient from time 24 ps to 28 ps and tends to possess the stable configuration in this period of time. For the podocin all mutations mutant model-boerhavinone E complex, the temperature graph of the complex shows slanted downwards gradient from time 26 ps to 28 ps and tends to possess the stable configuration in this period of time. For the podocin D160G mutant model-boerhavinone E complex, a steep downward gradient from time 22 ps to 24 ps, and a stable configuration from time 28 ps to 30 ps, hence the complex tends to attain the most stable configuration.

CHAPTER 6

CONCLUSION

In this study we have examined and investigated the interaction between the wild and mutant model of the two target renal proteins with the phytochemicals of three medicinal plants which are well-known for their therapeutic properties. We have also performed molecular simulations for both the wild and mutant model to understand and gain insights on the different conformational changes and the entire dynamics and hydration of the mutant protein structure.

The docking study performed for gaining insights revealed that the interaction energy for the wild type of nephrin protein is -26.67 kcal/mol, for the mutant type of nephrin protein is -28.06 kcal/mol, for the mutant type_P368S of nephrin protein is -25.64 kcal/mol, for the wild type of podocin protein is -18.94 kcal/mol, mutant type of podocin protein is -30.19 kcal/mol, for the mutant type_D160G of podocin protein is -30.46 kcal/mol respectively. In the wild model of nephrin protein, there exists one conventional hydrogen bond with threonine:416 and two hydrophobic pi-pi stacked with tryptophan:378, when interacting with boerhavinone E but there is no presence of hydrogen bond in the mutant models of nephrin protein with all the mutations present together but there is a presence of conventional hydrogen bond with arginine:407, one pi-alkyl bond with leucine:376 and one alkyl bond with methionine:384. However, the individual mutation induced models shows very weak interaction with boerhavinone E rather shows a better interaction with isocitric acid and caffeic acid having five conventional hydrogen bonds with valine:437, glutamine:442, glycine:469, glycine:470, asparagines:471, one carbon hydrogen-bond with serine:507, one carbon hydrogen bond with phenylalanine:404 and one conventional hydrogen bond with glycine:140. Similarly, for the wild model of podocin protein, it has one conventional hydrogen bond with glycine:140 when interacting with eucalyptol and the models with all the mutations induced together as well as separately does not show any interaction or presence of hydrogen bonds when interacting with eucalyptol. However, the mutant models

do show presence of conventional hydrogen bonds when interacting with boerhavinone C, isocitric acid and caffeic acid. The mutant model with all the mutations induced together shows three conventional hydrogen bonds with glutamic acid:198, alanine:200 and serine:201 with boerhavinone C, and the mutant_D160G with the highest docked score among mutant models have three conventional hydrogen bonds with arginine:146, glutamic acid:198 and asparagines:199 with boerhavinone C. Based on the results, we can say that the compound boerhavinone C reacts with mutation D160G model of podocin protein as well as wild model of podocin protein, similarly, the compounds isocitric acid and boerhavinone E reacts with the mutant_P368S model and wild model of nephrin protein respectively.

The simulation study of these selected compounds reveals that the total energy of the selected mutant models is lesser than the wild models, and hence suggests the fact that the mutant models are less stable, however, there exists very small variations in the energy which also suggests that the phytochemicals react almost in the same magnitude with the mutant and wild models. Therefore, from these results, we can conclude that the phytochemicals boerhavinone E, boerhavinone C and isocitric acid may prove to have the potential to be considered as drugs for the treatment and therapy of nephritic syndrome in the future.

CHAPTER 7

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