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Boerhaavia diffusa Linn. (Mookiratai) in the Treatment of Chronic Kidney Disease (CKD): A Network Pharmacology based Approach and Molecular Docking Studies

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ABSTRACT: CKD is global public health concern with more than 1 million cases per year in India. It is a condition in which Kidneys are damaged and causes edema. *Boerhaavia diffusa* is a species of flowering plant belonging to the family of Nyctaginaceae. It is well known as Mookiratai, Punarnava, Putpagam, Rathaputpika. It is traditionally given for edema, intestinal colic, kidney disorders, cough, haemorrhoids etc. This investigation will look into the mechanics of *Boerhaavia diffusa* on CKD through network pharmacology and molecular docking by identifying the compounds of the herb and relevant targets related to CKD which has highest binding affinity by protein ligand interaction. Multiple bioinformatics tools and online databases were used to obtain relevant targets of *Boerhaavia diffusa* on CKD. In the study of molecular docking, Autodock 2.4.2 software was used to determine the binding mode and interaction between the key active compounds of *Boerhaavia diffusa* and hub proteins. The molecular docking showed that Boerhavinone O, sitosterol were the key compounds which showed highest binding affinity against the ligand model p53, ABCG2, BACE1. The results predicted and verified the potential targets of *Boerhaavia diffusa* on CKD from a holistic perspective and paved way for further preclinical and clinical studies of BD (*Boerhaavia diffusa*). BD can become novel promising Siddha drug in the treatment of CKD.

Keywords: *Boerhaavia diffusa*, CKD, Molecular docking, Boerhavinone, Sitosterol, ABCG2, p53, BACE1.

INTRODUCTION

CKD is a global health concern and over 700 million cases have been reported. Many are unaware of the condition because they remain asymptomatic until the disease is near end stage. Over 70% of cases of late-stage CKD (stage 5 CKD and ESKD) are brought on by vascular disease or hypertension. Even if the underlying cause can be found, treated, or removed, CKD typically results in a steady deterioration in kidney function. In order to preserve overall homeostasis, nephron destruction causes compensatory hypertrophy and supranormal GFR of the surviving nephrons. Therefore, the serum creatinine is an insensitive marker for early renal injury and scarring even in the presence of a considerable loss of renal mass and may continue to be reasonably normal. Additionally, compensatory hyperfiltration damages the remaining nephrons from overuse, which in turn results in progressive glomerular sclerosis and interstitial fibrosis. Current treatment protocol involves lowering the blood pressure, uric acid and reducing proteinuria, these will not prevent the progression of renal impairment in CKD (Maxine and Papadakis 2020). Currently, over 2.6 million kidney transplants are being done and the rates will go higher in the coming up years.

Traditional Siddha Medicine (TSM) can treat CKD without much complications and side effects. It is one of the oldest systems of medicine which developed 10,000 years ago. Many research papers have been published depicting the effectiveness of *Boerhaavia diffusa* in the treatment of chronic kidney disease. This particular drug possesses rejuvenating capacity and helps in the formation of new cells and prevents deterioration of existing cells. It has anti-diabetic and diuretic properties (Mahesh, 2012). It can help in the treatment of kidney disorders like irregular blood pressure and diuresis. The aqueous extract is widely been used in the management of nephrotic syndrome.

In this study, a network pharmacology-based approach was employed to comprehend how drugs interact with various targets and what effects they have on the respective targets (Sun, 2022). In recent medical advancements network pharmacology plays a vital role in the holistic view of Siddha medicine. Active components of the drug have been identified and its mechanism of action in the required target has been investigated. Further docking studies are carried out in order to confirm interaction patterns and docking scores.

MATERIALS AND METHODS

Boerhaavia diffusa is a species of flowering plant belonging to the family of Nyctaginaceae. This separate drug possesses actions such as diuretic, expectorant, laxative, refrigerant, anthelmintic, and emetic (in large doses) (Mudhaliar, pp. 482-484).

Network Pharmacology Analysis. *Acquiring of Active Compounds and Related Targets in Boerhaavia diffusa*

Compounds of **Boerhaavia diffusa** are Boerhaavia acid, is oflavonoids (rotenoids), Punarnavine, sitosterol, Boeravinone, palmitic acid, steroid (ecdysteroid), lignan glycosides, and esters of sitosterol.

(Pubchem, 2004) PubChem Swiss Target Prediction databases were utilized to identify the targets corresponding to each active component of *Boerhaavia diffusa*.

Fishing for Candidate Targets of Boerhaavia diffusa in treating CKD

Targets related to CKD were acquired from Gene Cards, OMIM (University) databases. All the collected targets of CKD and *Boerhaavia diffusa* were then docked to determine the binding affinity and ligand efficacy between active components of *Boerhaavia diffusa* and target proteins. ABCG2, p53, and BACE1 were identified as target proteins having the highest interaction with the ligand model Boeravinone O and Sitosterol (Chandran, 2017).

Protein-ligand docking

Protein preparation. Protein Data Bank (PDB) (Table 1) was used to retrieve the crystal structures of p53 [PDB ID- 1TUP], ABCG2 [PDB ID- 6VXF], and BACE1 [PDB ID- 3R1G], which were then cleaned up and converted into the respective PDBQT formats.

Table 1: Target proteins and their respective PDB ID.

Target proteins	PDB ID
P53	1TUP
ABCG2	6VXF
BACE1	3R1G

Ligand preparation. Molinspiration Molecular Viewer (Molinspiration, 1986) was used to obtain 2-D and 3-D structures of the ligand molecule. It has a collection of molecules encoded with SMILE (National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 71662493, Boeravinone O. Retrieved November 23, 2022) (National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 222284, Beta-Sitosterol. Retrieved November 23, 2022), molecular properties and bioactive scored of the ligand Boeravinone O and Sitosterol. It was downloaded in PDBQT format and further used in docking studies (Operations).

Molecular Docking. Molecular interaction analysis done by using Autodock 4.2.6 (Bikadi, 2009) Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out for the phytochemicals Boeravinone O, Sitosterol against target protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris, 1998). Affinity (grip) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (Solis, 1981). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. Visualization of protein-ligand docking interactions were seen using Biovia Discovery Studio Visualizer.

Table 2: Ligand Properties of the compounds selected for docking analysis.

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Boeravinone O	328.27	$C_{17}H_{12}O_7$	3	7	1
Sitosterol	414.7	$C_{29}H_{50}O$	1	1	6

Table 3a: Hydrogen bond interaction of Boeravinone O against ABCG2- PDB- 6VXF.

ABCG2		Boeravinone O	Distance (Å)	Docking energy (Kcal/Mol)
Residue	Atom			
A:GLY422:N - A:SER420:O	N	O	2.97497	**-5.01**
A:GLN424:N - A:GLY422:O	N	O	3.17629	
A:ASN425:N - A:GLY422:O	N	O	3.13643	
A:ARG426:N - A:GLY422:O	N	O	2.87276	
A:THR598:N - A:ASN596:O	N	O	3.16955	

A:THR607:OG1 - A:THR607:O	OG1	O	3.01153	
B:ASN604:N - B:CYS603:SG	N	SG	2.90292	
B:ASN604:N - B:ASN604:OD1	N	OD1	2.474	
B:ASN604:ND2 - A:TYR605:O	ND2	O	2.76865	
A:ILE423:N - :UNL1:O	N	O	3.15785	

Table 3b: Hydrogen bond interaction of Sitosterol against ABCG2- PDB- 6VXF.

ABCG2		Sitosterol	Distance (Å)	Docking energy (Kcal/Mol)
Residue	Atom			
A:THR642:N - A:VAL638:O	N	O	2.9059	**-7.48**
A:THR642:OG1 - A:VAL638:O	OG1	O	2.43329	
A:TYR645:N - A:LEU641:O	N	O	2.86375	

Table 3c: Hydrogen bond interaction of Boeravinone O against BACE1- PDB- 3R1G.

BACE1		Boeravinone O	Distance (Å)	Docking energy (Kcal/Mol)
Residue	Atom			
B:SER96:OG - B:ASP93:OD2	O	O	2.56538	**-5.19**
B:THR133:OG1 - B:THR133:O	O	O	2.79697	
B:THR292:OG1 - B:ASP289:OD2	O	O	2.55885	

Table 3d: Hydrogen bond interaction of Sitosterol against BACE1- PDB- 3R1G.

BACE1		Sitosterol	Distance (Å)	Docking energy (Kcal/Mol)
Residue	Atom			
H:ASP86:N - H:ALA84:O	N	O	2.95898	**-5.3**
H:THR87:N - H:ALA84:O	N	O	3.0122	
H:THR87:OG1 - H:ALA84:O	O	O	3.00278	

Table 3e: Hydrogen bond interaction of Boeravinone O against p53- PDB- 1TUP.

P53		Boerhavinone O	Distance (Å)	Docking energy (Kcal/Mol)
Residue	Atom			
A:PHE113:N - :UNL1:O	N	O	2.72875	**-4.4**
A:ASN131:ND2 - A:TYR126:OH	N	O	2.59384	
A:ASN131:ND2 - A:ASN131:O	N	O	2.92765	

Table 3f: Hydrogen bond interaction of Boeravinone O against p53- PDB- 1TUP.

P53		Sitosterol	Distance (Å)	Docking energy (Kcal/Mol)
Residue	Atom			
C:ASN131:HD21 - C:TYR126:OH	H	O	1.50762	**-7.9**
C:SER269:HN - :UNL1:O	H	O	2.13788	

Fig. 1a. 3D- Structure of ATP-binding cassette family 2 (ABCG2) - PDB ID 6VFX.

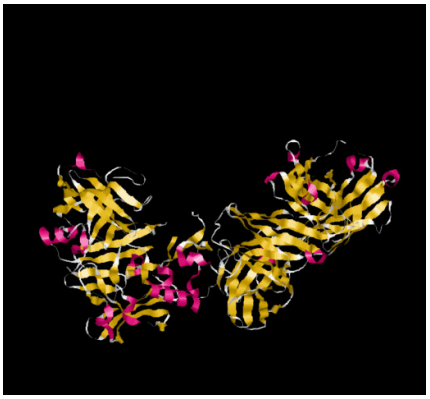


Fig. 1b. 3D- Structure of beta-site APP-cleaving enzyme 1(BACE1) - PDB ID 3R1G.

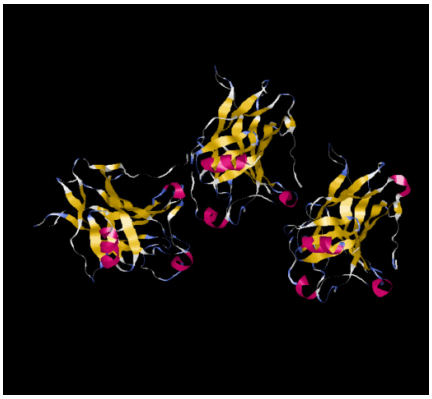


Fig. 1c. 3D- Structure of Tumour protein (p53) - PDB ID 1TUP.

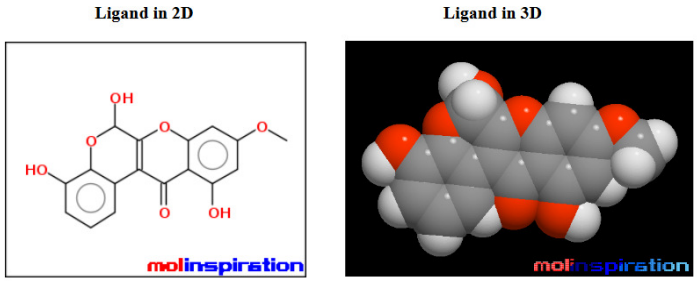


Fig. 2a. Boeravinone O.

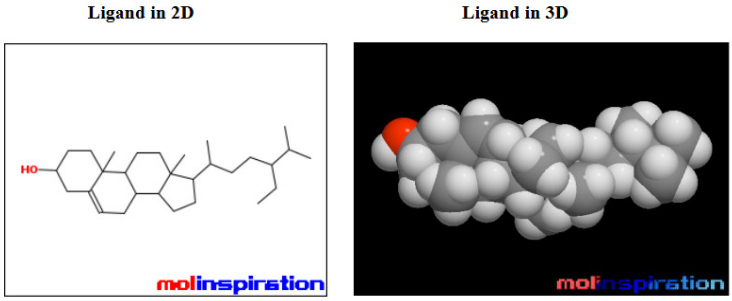
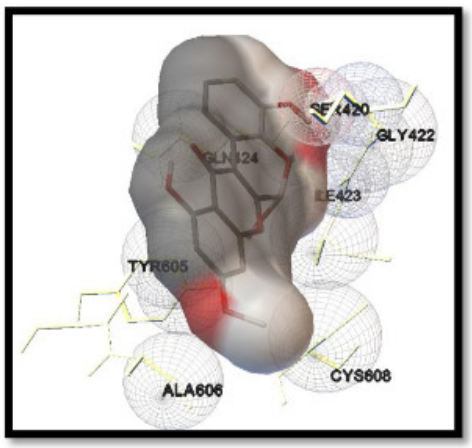
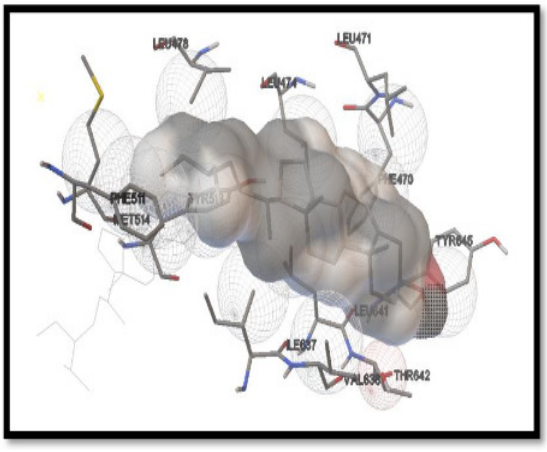


Fig. 2b. Sitosterol.

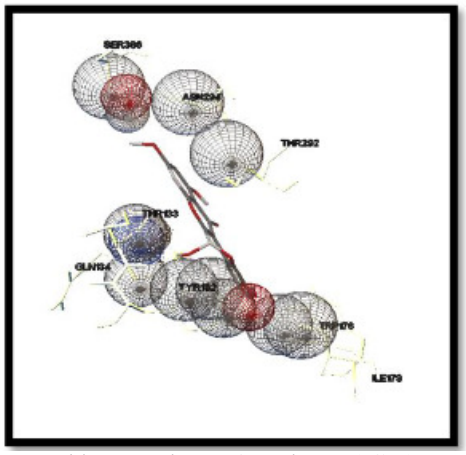
Fig. 2. 2D and 3D structure of selected ligands (Molinspiration, 1986).



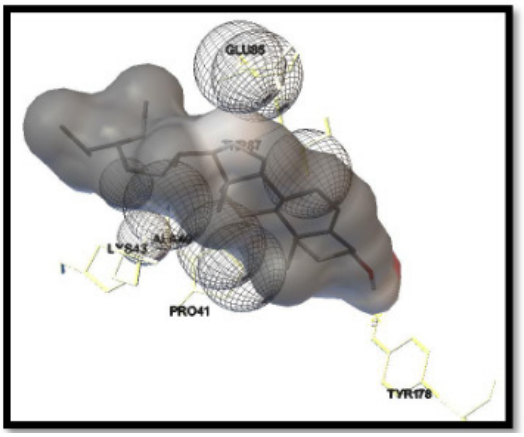
(a) Boeravinone O against ABCG2



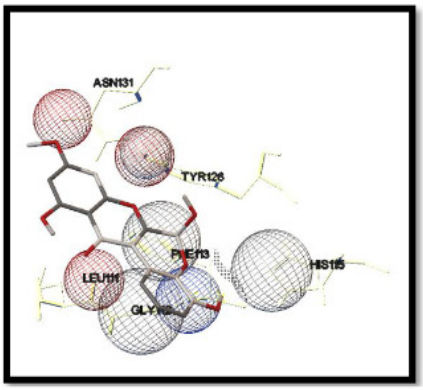
(b) Sitosterol against ABCG2



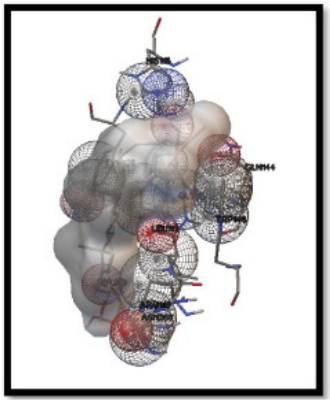
(c) Boeravinone O against BACE1



(d) Sitosterol against BACE1

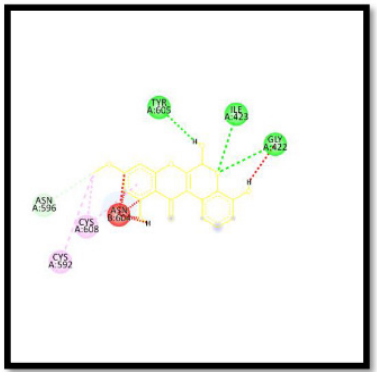


(e) Boeravinone O against p53

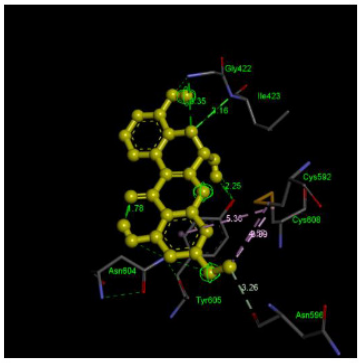


(f) Sitosterol against p53

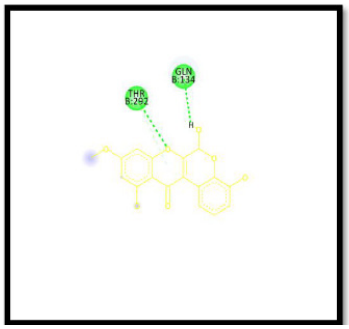
Fig. 3. Docking results with conformation info in Auto Dock 4.2.6.



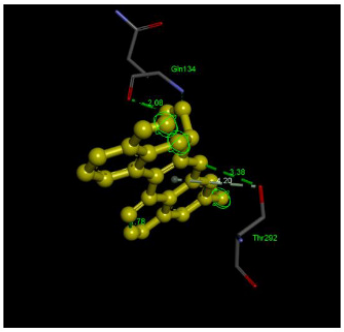
(a) Boeravinone O against ABCG2 2D structure



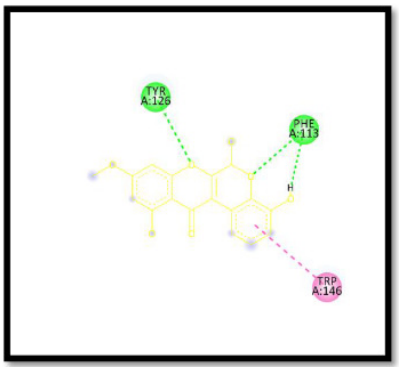
(b) Boeravinone O against ABCG2 3D structure



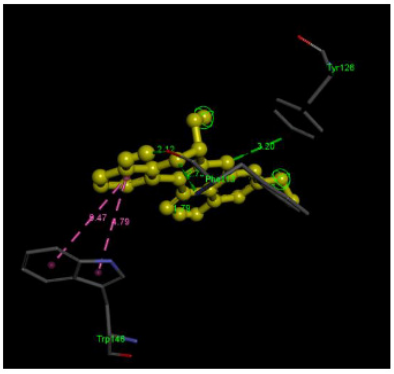
(c) Boeravinone O against BACE1 2D structure



(d) Boeravinone O against BACE1 3D structure

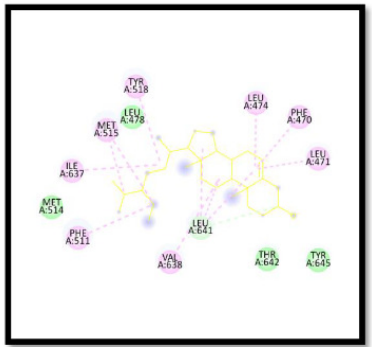


(e) Boeravinone O against p53 2D structure

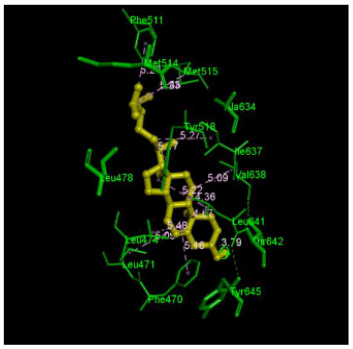


(f) Boeravinone O against p53 3D Structure.

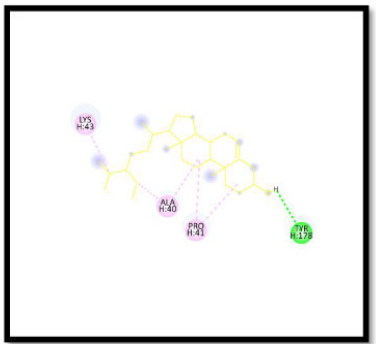
Fig. 4. Visualization of protein-ligand docking interactions using Biovia discovery studio visualizer (Boeravinone O and respective target proteins (Systemes, 2002 - 2024).



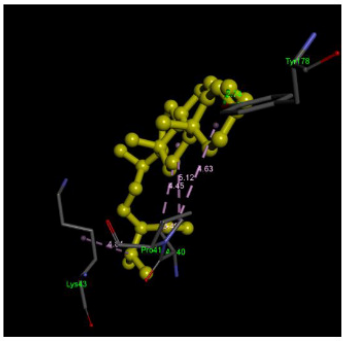
(a) Sitosterol against ABCG2 2D structure



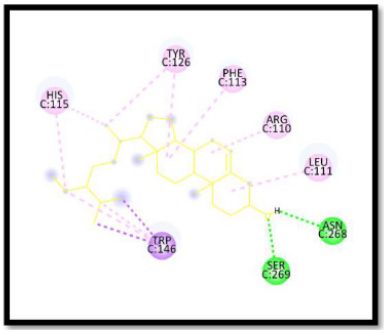
(b) Sitosterol against ABCG2 3D structure



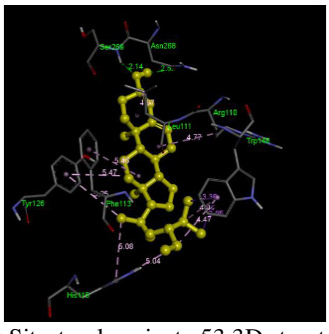
(c) Sitosterol against BACE1 2D structure



(d) Sitosterol against BACE1 3D structure



(e) Sitosterol against p53 2D structure



(f) Sitosterol against p53 3D structure

Fig. 5. Visualization of protein-ligand docking interactions using Biovia discovery studio visualizer (Sitosterol and respective target (Systèmes, 2002 - 2024).

RESULTS AND DISCUSSION

Boerhaavia diffusa is used to treat CKD, hence molecular docking studies for the drug's primary phytochemicals and target proteins were done. Table 2 provides the results of these ligands' binding affinities for the corresponding targets.

Among all docked components Sitosterol shows highest binding affinity of -7.9 kcal/mol for p53 and -7.48 kcal/mol for ABCG2 respectively. However it showed reduced expression against BACE1 with the binding affinity of -5.3. Boeravinone O possess higher binding affinity compared to Sitosterol for BACE1 with -5.19 kcal/mol. Sitosterol interacted with 8 amino acid residues and with lowest distance of bond formation with ASN 268, TYR 126, SER 269 respectively for p53, interacted with 13 amino acid residues and with lowest distance of bond formation with THR 642, TYR 645, VAL 638 respectively for ABCG2, interacted with 4 amino acid residues and with lowest distance of bond formation with ALA 84, THR 87 respectively for BACE1 Fig. 4 (Halgren, 1998). Boeravinone O interacted with 3 amino acid residues and with lowest distance of bond formation with TYR 126 for p53, interacted with 7 amino acid residues and with lowest distance of bond formation with ASN 604, CYS 603, GLY 422, SER 420, ARG 426 respectively for ABCG2, interacted with 2 amino acid residues and with lowest distance of bond formation with THY292, ASP 289, SER 96 respectively for BACE1 (Fig. 5).

Table 4: Binding energy of lead components of *Boerhaavia diffusa* against respective targets.

Sr. No.	Compounds	Binding Free energy Kcal/ mol ABCG2	Binding Free energy Kcal/ mol BACE1	Binding Free energy Kcal/ mol p53
1.	Boeravinone O	-5.01	-5.19	-4.4
2.	Sitosterol	-7.48	-5.3	-7.9

CONCLUSIONS

Based on the outcomes of the computational study, it was determined that chemicals found in *Boerhaavia diffusa*, such as Boerhavinone O and sitosterol, significantly inhibit ABCG2 and thereby renal urate under secretion and induced hyperuricemia causing CKD can be prevented. BACE1, p53- it is a potential tumor suppressor and plays a critical role in AKI (Acute kidney injury) occurrence and progression of Chronic Kidney Disease (CKD). Sitosterol showed the highest binding affinity against ABCG2 and p53. However, it showed limited expression against BACE1. Boerhavinone O has the highest binding affinity against BACE1 followed by ABCG2 but has limited expression against p53. As there is no standard drug administered in the treatment of CKD, the Binding affinity of the ligand and target are studied and concluded. Further toxicity, Pre-clinical, and clinical studies are to be done to prove its efficacy against potential targets. Thereby formulations including *Boerhaavia diffusa* may have promising activity against CKD.

FUTURE SCOPE

By 2040, chronic kidney disease (CKD) will rank as the fifth leading cause of mortality globally. Premature mortality is where it has the biggest effect, but there are also a lot more people with kidney failure who need renal replacement therapy (RRT). Due to the lack of kidney donors and the poor results of peritoneal and bone haemodialysis, current RRT is inadequate (Copur *et al.,* 2023). *Boerhaavia diffusa* has already been proved to treat even cerebral edema caused by chronic kidney disease. This plant is easily available and is cost effective. Plant's decoction can be easily prepared and ingested by the patients. This medication doesn't cause any adverse effects and can show good prognosis. Further clinical trials should be done to prove its actual efficacy in treating or reducing the progression of the disease. This study is preliminary to manifest drug's potency in treating the disease. TSM (Traditional Siddha Medicine) consists of many herbal and mineral formulations which are derived from literatures. Many undiscovered potency of drugs are to be proven in treating various kinds of ailments.

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