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IN SILICO EVALUATION OF PHYTOCONSTITUENTS OF SANJEEVI CHOORNAM THROUGH MOLECULAR DOCKING AND ADME ANALYSIS AGAINST PROTEINURIA

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ABSTRACT

Background and Objective: Proteinuria is a major clinical manifestation of renal dysfunction and an important predictor of chronic kidney disease progression. Nephrin, a key structural protein of podocytes, and Angiotensin-Converting Enzyme (ACE), a crucial component of the renin–angiotensin system, play significant roles in the development of proteinuria. Sanjeevi Choornam, a traditional Siddha herbal formulation, has been used for the management of renal disorders. The present study aimed to evaluate the molecular docking interactions and ADME profiling of selected phytoconstituents of Sanjeevi Choornam against Nephrin and ACE using computational approaches. **Materials and Methods:** Selected phytoconstituents of Sanjeevi Choornam were subjected to molecular docking analysis against Nephrin and ACE target proteins using Auto Dock Vina. The binding affinities of the phytocompounds were compared with standard drugs Losartan for Nephrin and Enalapril for ACE. Molecular interactions, binding energies, and amino acid residues involved in ligand–protein interactions were analyzed. Pharmacokinetic and drug-likeness properties were evaluated using SwissADME. **Results:** The selected phytoconstituents exhibited significant binding affinity towards both Nephrin and ACE proteins. Several compounds demonstrated stronger or comparable binding affinities compared to the standard drugs Losartan and Enalapril. The docked compounds formed stable hydrogen bonds and hydrophobic interactions with key active-site residues, indicating potential nephroprotective activity. ADME analysis revealed favorable pharmacokinetic properties, including good gastrointestinal absorption, acceptable bioavailability, and acceptable drug-likeness characteristics. **Conclusion:** The findings suggest that the phytoconstituents present in Sanjeevi Choornam possess promising nephroprotective potential through effective interactions with Nephrin and ACE, key molecular targets involved in proteinuria. The observed docking scores, comparable to those of Losartan and Enalapril, together with favorable ADME properties, support the therapeutic potential of Sanjeevi Choornam in the management of proteinuria. Further experimental and clinical studies are required to validate these findings.

KEYWORDS: Proteinuria, Sanjeevi Choornam, Molecular Docking, ADME Profiling, Nephrin, ACE, Losartan, Enalapril, Siddha Medicine.

INTRODUCTION

Proteinuria is one of the most important clinical manifestations of renal dysfunction and serves as an important marker for the progression of chronic kidney disease (CKD). It is characterized by the abnormal excretion of proteins in urine due to impairment of the glomerular filtration barrier. Persistent proteinuria contributes to progressive renal damage through mechanisms involving inflammation, oxidative stress, podocyte injury, and glomerulosclerosis. Among the various molecular factors involved in renal function, Nephrin and Angiotensin-Converting Enzyme (ACE) play crucial roles in maintaining glomerular integrity and regulating renal hemodynamics. Alterations in Nephrin expression led to podocyte dysfunction and increased protein leakage, whereas overactivation of ACE promotes intraglomerular hypertension and progressive renal injury through the renin–angiotensin system.

In conventional medicine, ACE inhibitors and angiotensin receptor blockers such as Enalapril and Losartan are widely used to reduce proteinuria and slow the progression of kidney disease. However, long-term treatment may be associated with adverse effects and variable therapeutic responses, highlighting the need for safer and more effective alternative therapies.

According to Siddha literature, renal disorders characterized by excessive, turbid, frothy, or abnormal urination are broadly classified under Mega Neer (Meham) disorders. Siddha texts describe Mega Neer as a condition resulting from derangement of the three humors (Vali, Azhal, and Iyyam), leading to abnormalities in urine quantity, quality, and frequency. Clinical manifestations described under Mega Neer, such as excessive urination, weakness, edema, and urinary abnormalities, closely resemble the symptoms observed in chronic renal disorders and proteinuria. Siddha management aims to restore the balance of the three humors, improve urinary function, strengthen renal tissues, and prevent disease progression.

Sanjeevi Choornam is a classical Siddha herbal formulation traditionally indicated for urinary and renal disorders. The formulation contains medicinal herbs possessing nephroprotective, anti-inflammatory, antioxidant, and diuretic properties, which may help protect the glomerular filtration barrier and improve renal function. Despite its traditional therapeutic use, the molecular mechanisms underlying its renoprotective activity have not been scientifically explored.

Recent advances in computational drug discovery have enabled the use of molecular docking and ADME profiling to evaluate the interaction of phytoconstituents with disease-associated molecular targets. Molecular docking predicts the binding affinity and interaction patterns between bioactive compounds and target proteins, while ADME analysis assesses pharmacokinetic and drug-likeness properties essential for drug development.

Therefore, the present study was undertaken to investigate the molecular docking interactions and ADME profiling of selected phytoconstituents of Sanjeevi Choornam against the key proteinuria-associated targets Nephrin and ACE. The docking performance of the phytoconstituents was compared with the standard drugs Losartan and Enalapril to evaluate their potential nephroprotective activity and provide scientific evidence for the traditional use of Sanjeevi Choornam in the management of Mega Neer-related renal disorders.

MATERIALS AND METHODS

Collection of Phytoconstituents

The phytoconstituents present in Sanjeevi Choornam were identified through an extensive review of Siddha literature and published scientific reports. The major bioactive compounds selected for the present study were retrieved from the pubchem.ncbi.nlm.nih.gov in Structure Data File (SDF) format. The selected compounds were prepared and energy minimized before molecular docking analysis.

Ingredients of Sanjeevi Choornam

Table 1: Ingredients of Sanjeevi Choornam.

Ingredients	Family	Active ingredients	
Tinospora cordifolia	Menispermaceae	Berberin	Antioxidant and anti-inflammatory effects; reduces oxidative stress and protects renal tubular cells from injury.
Myristica fragrans	Myristicaceae	Myristicin	Antioxidant activity reduces lipid peroxidation and inflammatory damage in kidney tissues.
Myristica fragrans	Myristicaceae	Macelignan	Scavenges reactive oxygen species and may attenuate inflammatory pathways involved in renal injury.
Piper cubeba	Piperaceae	Cubebin	Anti-inflammatory and antioxidant properties; may help reduce renal oxidative stress and improve renal function markers.
Elettaria cardamomum	Zingiberaceae	1,8-Cineole	Mild diuretic activity, antioxidant action, and reduction of inflammatory mediators in renal tissues.
Syzygium aromaticum	Myrtaceae	Eugenol	Potent antioxidant and anti-inflammatory effects; protects against nephrotoxicity and oxidative renal damage.
Papaver somniferum	Papaveraceae	Linoleic acid	Antioxidant properties may help maintain renal cellular integrity and reduce oxidative stress.
Abies webbiana	Pinaceae	α-Pinene	Anti-inflammatory and antioxidant effects that may contribute to protection against renal inflammation.
Quercus infectoria	Fagaceae	Gallic acid	Strong antioxidant and anti-inflammatory actions; may reduce renal oxidative injury and inflammatory responses

Preparation of Ligand

The three-dimensional (3D) structures of the selected phytoconstituents were downloaded from the PubChem database and converted into Protein Data Bank (PDB) format using Open Babel software. Energy minimization was performed to obtain stable conformations suitable for docking studies. The prepared ligands were saved in PDBQT format for AutoDock Vina analysis.

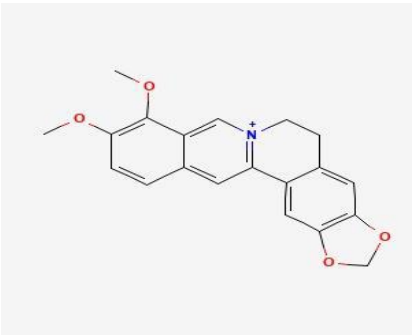
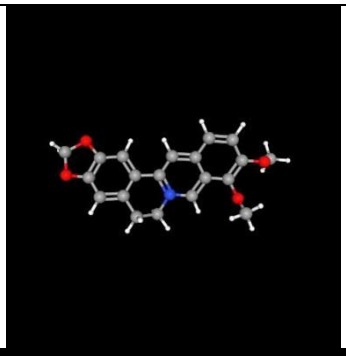
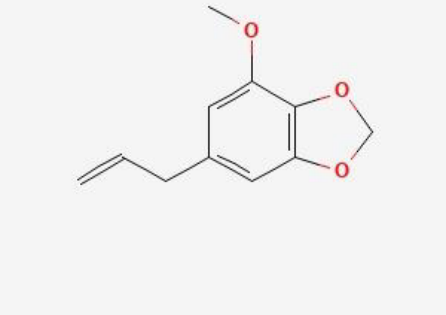
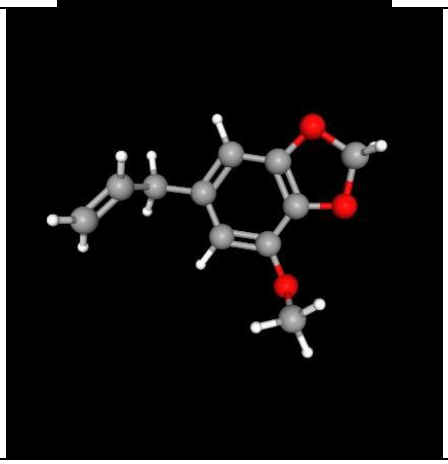
The identified phytochemicals along with their Molecular weight, Molecular formula, H-bond donor, H-bond acceptor, Rotatable bonds were listed in table 2.

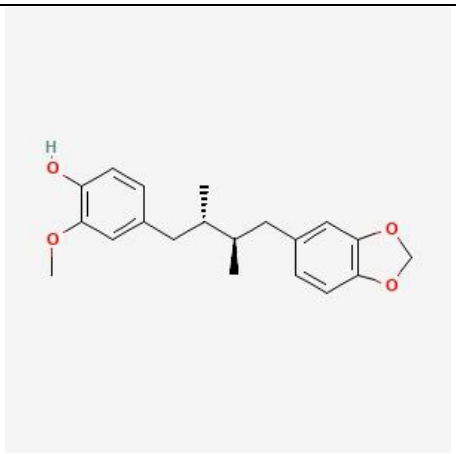
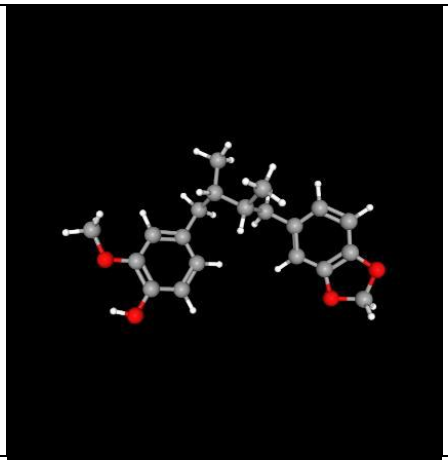
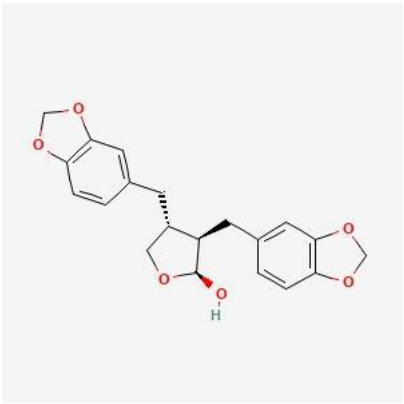
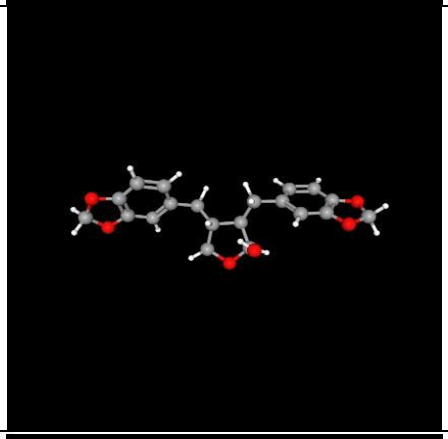
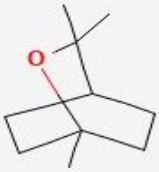
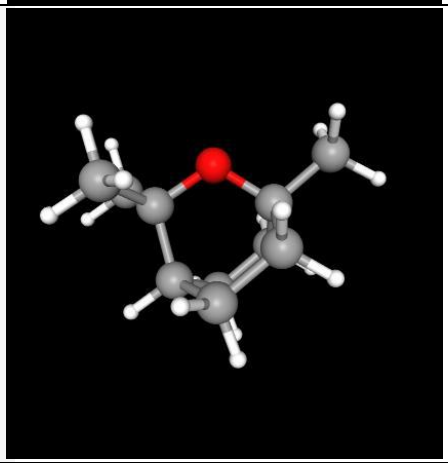
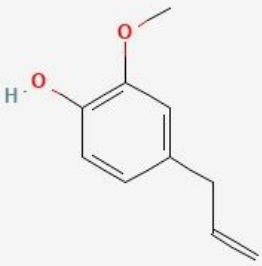
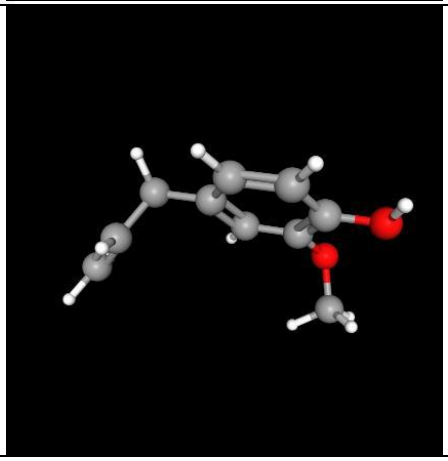
Table 2: Chemical properties of selected Ligands.

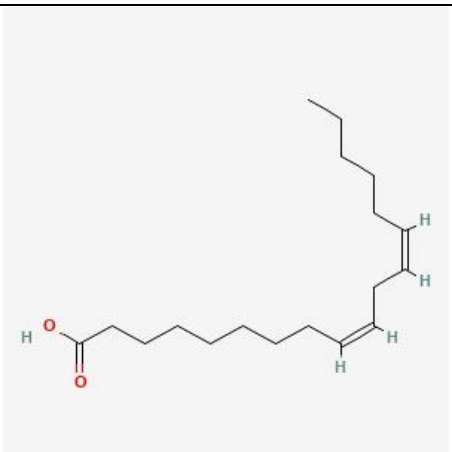
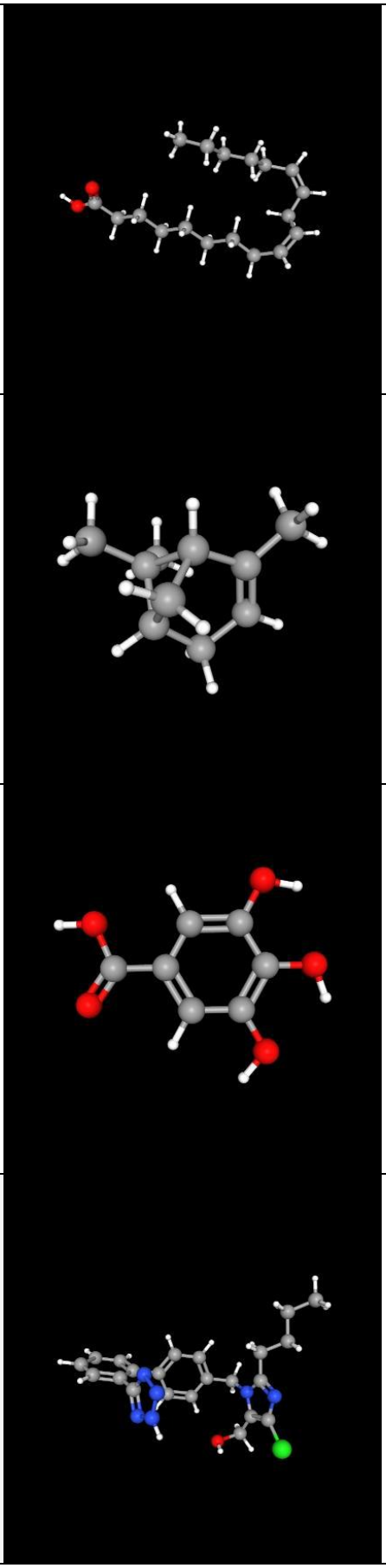
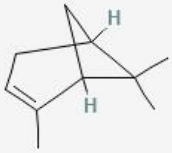
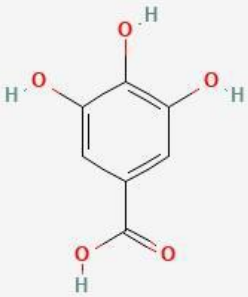
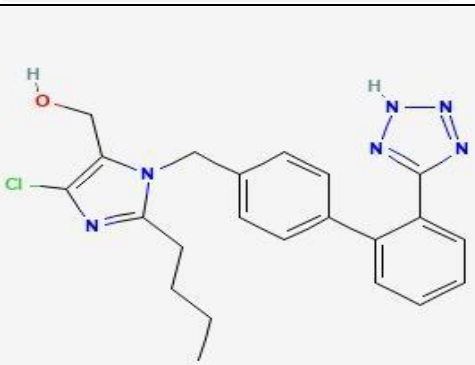
Compound	Molecular Weight g/mol	Molecular formula	H- bond donor	H- bond acceptor	Rotatable bonds
Berberin	336.4	$C_{20}H_{18}NO_4$	0	4	2
Myristicin	192.21	$C_{11}H_{12}O_3$	0	3	3
Macelignan	328.4	$C_{20}H_{24}O_4$	1	4	6
Cubebin	356.4	$C_{20}H_{20}O_6$	1	6	4
1,8-Cineole	154.25	$C_{10}H_{18}O$	0	1	0
Eugenol	164.20	$C_{10}H_{12}O_2$	1	2	3
Linoleic acid	280.4	$C_{18}H_{32}O_2$	1	2	14
α-Pinene	136.23	$C_{10}H_{16}$	0	0	0
Gallic acid	170.12	$C_7H_6O_5$	4	5	1
Losartan	422.9	$C_{22}H_{23}ClN_6O$	2	5	8
Enalapril	376.4	$C_{20}H_{28}N_2O_5$	2	6	10

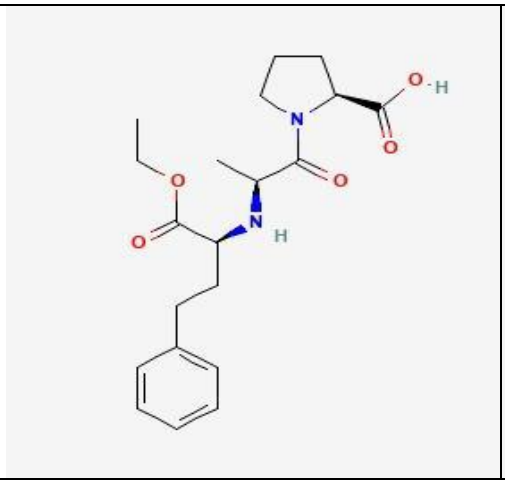
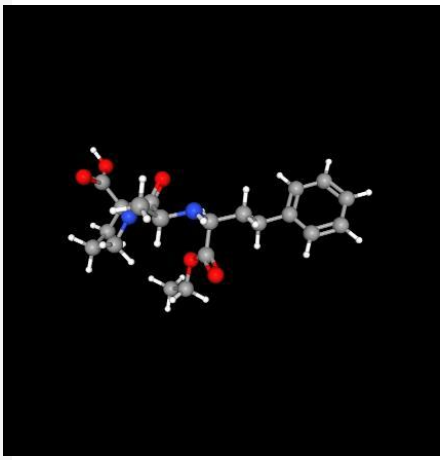
Each selected phytochemical was prepared for docking by obtaining its 2D and 3D structures from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and converted to PDB format, followed by energy minimization to ensure stable conformations and reduced steric hindrance. Each ligand was then parameterized with appropriate partial charges and rotatable bonds to enable flexible interactions with the target protein. The structured of the ligands are shown in Table 3.

Table 3: 2D and 3D structure of the selected Ligands.

Compound	2D Structure	3D structure
Berberin		
Myristicin		

Macelignan		
Cubebin		
1,8-Cineole		
Eugenol		

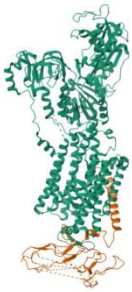
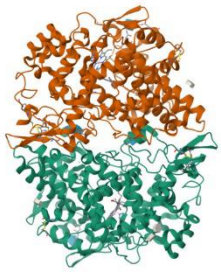
Linoleic acid		
α-Pinene		
Gallic acid		
Losartan		

Enalapril		

Preparation of Target

The target proteins were selected based on Swiss target prediction (http://www.swisstargetprediction.ch/index.php). The predicted target proteins were downloaded from RCSB PDB database (https://www.rcsb.org/). The x-ray diffraction structure of the different target proteins under study having resolution not less than 2 A^0 were used for the study (Table 4). Co-factors, ligands, water molecules, etc., were removed and converted into PDB format.

Table 4: Selected targets and their action.

Target protein	PDB ID	Structure	Role in Renal damage
Nephrin	5YLU		phrin is a key structural protein of the podocyte slit diaphragm. Reduced nephrin expression damages the filtration barrier and permits urinary loss of albumin, leading to proteinuria.
ACE	5IKR		ACE increases angiotensin II production, which elevates glomerular pressure and induces inflammation and podocyte injury, resulting in increased urinary protein excretion

Molecular Docking

Docking simulations were conducted using MGL AutoDock tools to evaluate the binding interactions between the target protein and each ligand. Molecular interaction analysis was performed using AutoDock 1.5.7 Morris et al. (2009) by following steps: Gasteiger partial charges were added to the ligand atoms. Nonpolar hydrogen atoms were merged, and rotatable bonds were defined. A grid box was centered on key active site residues to confine docking to relevant regions. Parameters, including binding affinity (ΔG), inhibition constant (Ki) and interaction surface, were calculated for each ligand. Docking was repeated using SwissDock Vina platform.

ADME and Drug-Likeness Prediction

The pharmacokinetic properties of the selected phytocompounds were evaluated using the SwissADME web server to determine their drug-likeness, absorption, distribution, metabolism, and excretion characteristics. Parameters including gastrointestinal absorption, blood–brain barrier permeability, cytochrome P450 inhibition, P-glycoprotein interaction, skin permeation, Lipinski's rule of five, bioavailability score, and medicinal chemistry properties were analyzed.

RESULTS AND DISCUSSION

The molecular docking study was performed to evaluate the binding affinity of the selected phytoconstituents of

Sanjeevi Choornam against the proteinuria-associated targets Nephrin and ACE. The docking results revealed significant interactions between the phytocompounds and the active sites of both target proteins. Lower binding energy values indicate stronger ligand–protein interactions and greater binding stability. The binding affinities of the phytocompounds were further compared with the standard drugs Losartan (Nephrin) and Enalapril (ACE).

Among the evaluated compounds, Cubebin exhibited the strongest interaction with ACE, showing a binding energy of −10.6 kcal/mol with 18 molecular interactions, which was considerably higher than the standard drug Enalapril (−8.5 kcal/mol). Similarly, Cubebin demonstrated a strong binding affinity against Nephrin with a docking score of −9.2 kcal/mol and 12 molecular interactions.

Macelignan also showed excellent binding affinity against Nephrin (−9.1 kcal/mol) and ACE (−8.9 kcal/mol), indicating stable interactions with the active amino acid residues of both proteins. Berberine exhibited docking scores of −8.9 kcal/mol against Nephrin and −9.5 kcal/mol against ACE, suggesting potent nephroprotective activity.

Myristicin demonstrated moderate binding affinity towards Nephrin and ACE with docking energies of −6.5 kcal/mol against both targets. Likewise, Eugenol, Gallic acid, Linoleic acid, α-Pinene, and 1,8-Cineole exhibited moderate interactions with the selected proteins. Although their binding energies were lower than those of Cubebin, Macelignan, and Berberine, these compounds may contribute synergistically to the overall therapeutic efficacy of the formulation.

The standard drug Losartan showed a docking score of −9.8 kcal/mol against Nephrin, while Enalapril exhibited a docking score of −8.5 kcal/mol against ACE. Interestingly, Cubebin demonstrated stronger binding affinity against ACE than Enalapril, whereas Macelignan and Berberine displayed binding energies approaching that of Losartan against Nephrin. These findings indicate that the phytoconstituents present in Sanjeevi Choornam may possess significant nephroprotective potential comparable to currently used therapeutic agents.

Role of Nephrin and ACE in Proteinuria

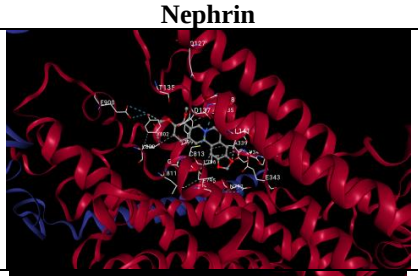
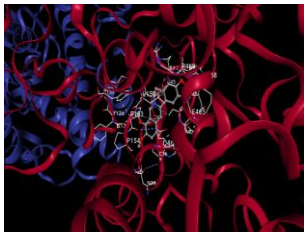
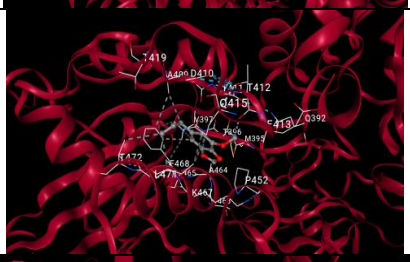
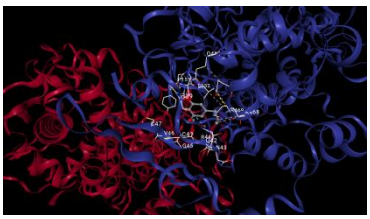
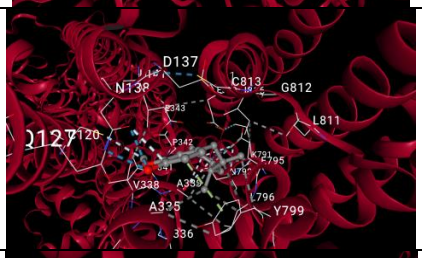
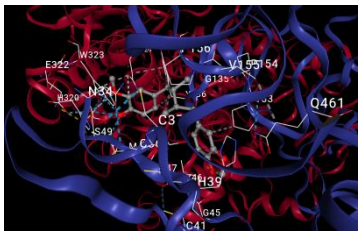
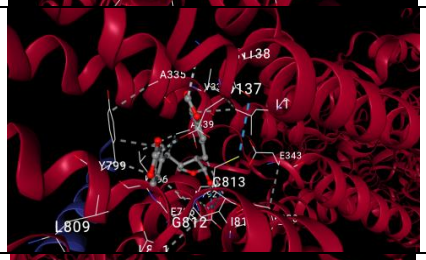
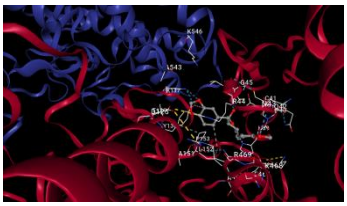
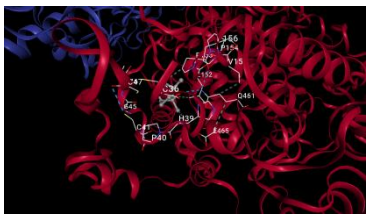
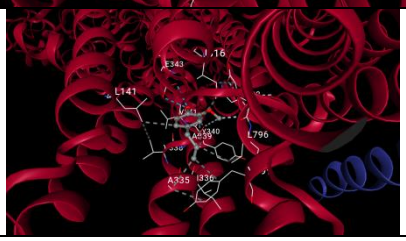
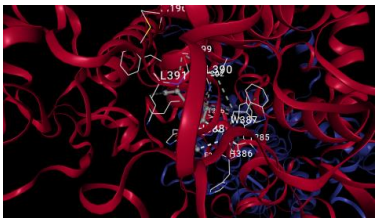
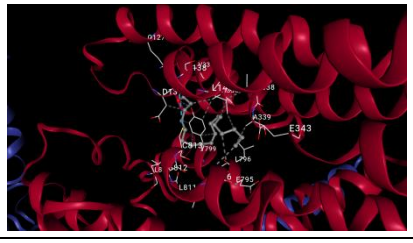
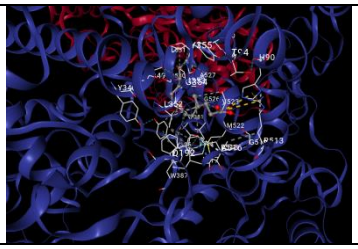
Nephrin is an essential transmembrane protein of the podocyte slit diaphragm that maintains the integrity of the glomerular filtration barrier. Reduced Nephrin expression or structural dysfunction leads to increased glomerular permeability and urinary protein loss. Therefore, compounds demonstrating strong interactions with Nephrin may help preserve podocyte function and reduce proteinuria.

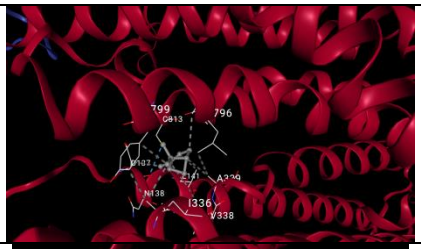
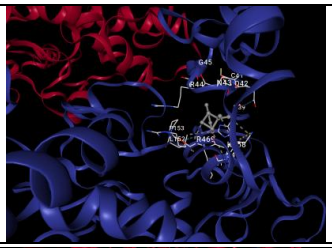
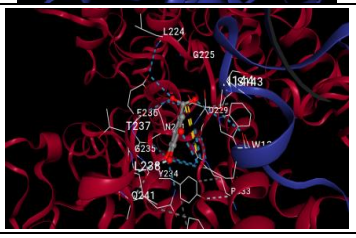
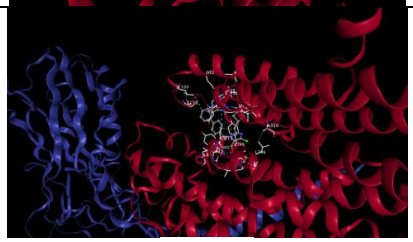
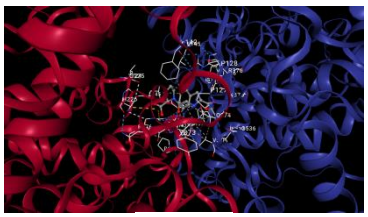
ACE is a key component of the renin–angiotensin–aldosterone system (RAAS). Increased ACE activity elevates angiotensin II levels, resulting in glomerular hypertension, inflammation, fibrosis, and progressive renal damage. Inhibition of ACE remains one of the most effective therapeutic strategies for reducing proteinuria and slowing chronic kidney disease progression. The strong interaction of Cubebin, Berberine, and Macelignan with ACE suggests their potential to modulate RAAS-mediated renal injury.

Table 5: Binding energies of different compounds.

Compound	Nephrin		ACE	
	No. of Interactions	Highest Binding Free Energy (Kcal/ mol)	No. of Interactions	Highest Binding Free Energy Kcal/ mol
Berberin	18	-8.9	10	-9.5
Myristicin	19	-6.5	20	-6.5
Macelignan	13	-9.1	20	-8.9
Cubebin	20	-9.2	18	-10.6
1,8-Cineole	20	-6.0	20	-5.6
Eugenol	20	-6.0	20	-6.4
Linoleic acid	19	-6.8	20	-6.7
α-Pinene	10	-5.8	20	-5.6
Gallic acid	19	-6.2	18	-6.4
Standard drug	20	-9.8(Losartan)	19	-8.5 (Enalapril)

Table 6: Docking interactions of Different compounds.

Compound	Nephrin	ACE
Berberin		
Myristicin		
Macelignan		
Cubebin		
1,8-Cineole		
Eugenol		
Linoleic acid		

α-Pinene		
Gallic acid		
Standard drug	 Losartan	 Enalapril

ADME PROPERTIES

Table 7: ADME properties of Different compounds.

Compounds	GI absorption	BBB permeant	P-gp	CYP1A2 inhibitors	CYP2C19 inhibitors	CYP2C9 inhibitors	CYP2D6 inhibitors	CYP3A4 inhibitors	Log Kp (cm/s)
Berberin	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-5. 78
Myristicin	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-5. 39
Macelignan	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-4. 64
Cubebin	High	Yes	No	No	No	No	No	Yes	-6.07
1,8-Cineole	High	Yes	No	No	No	No	No	No	-5. 30
Eugenol	High	Yes	No	Yes	Yes	Yes	No	Yes	-5. 69
Linoleic acid	High	Yes	No	No	No	No	No	No	-3. 05
A-Pinene	Low	Yes	No	Yes	No	No	No	Yes	-3. 95
Gallic acid	High	No	No	No	No	No	No	No	-6. 84
Losartan	High	No	Yes	No	No	Yes	No	Yes	-5. 83
Enalapril	High	No	No	No	No	No	No	No	-8. 65

ADME profiling demonstrated favorable pharmacokinetic properties for most of the selected phytoconstituents. Berberine, Myristicin, Macelignan, Cubebin, 1,8-Cineole, Eugenol, Linoleic acid, and Gallic acid exhibited high gastrointestinal absorption, indicating good oral bioavailability potential. Only α-Pinene showed relatively low gastrointestinal absorption.

Most compounds demonstrated blood–brain barrier permeability, while Gallic acid and Losartan were predicted to be non-BBB permeant. Cubebin showed a favorable safety profile with minimal CYP450 inhibition, reducing the possibility of metabolic drug interactions. Berberine, Macelignan, and Myristicin exhibited inhibition of multiple CYP450 enzymes, which may influence drug metabolism and require further investigation.

Drug-likeness

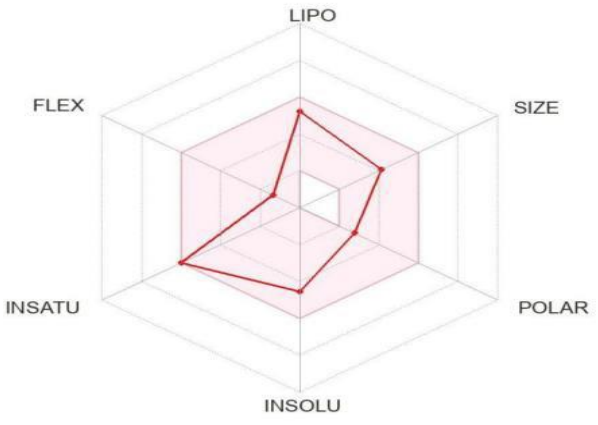
Table 8: Drug-likeness of the compounds.

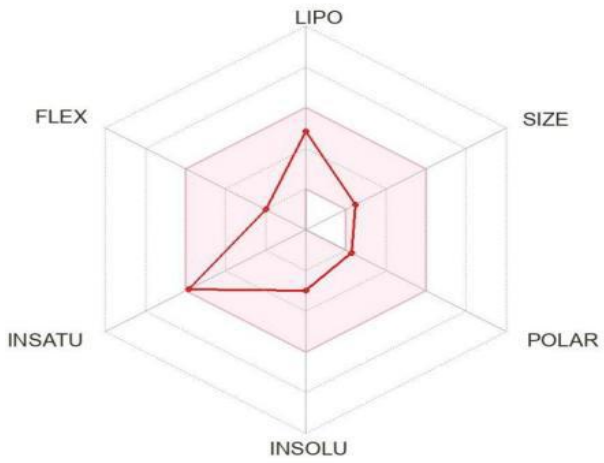
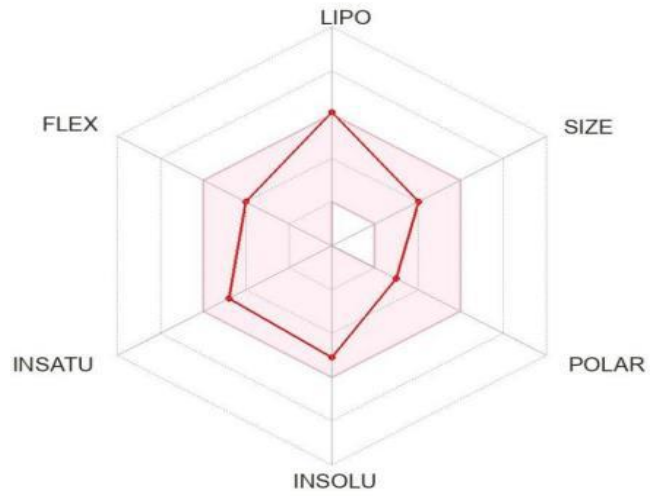
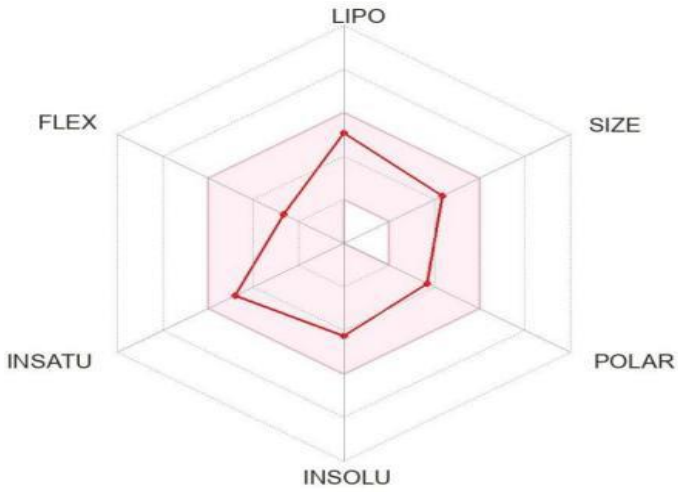
Compounds	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability
Berberin	Yes 0 violations	Yes	Yes	Yes	Yes	0.55
Myristicin	Yes 0 violations	Yes	Yes	Yes	No 1 violation	0.55
Macelignan	Yes 0 violations	Yes	Yes	Yes	No 1 violation	0.55
Cubebin	Yes 0 violations	Yes	Yes	Yes	Yes	0.55
1,8-Cineole	Yes 0 violations	No 1 violation	Yes	Yes	No 2 violations	0.55
Eugenol	Yes 0 violations	Yes	Yes	Yes	No 1 violation	0.55
Linoleic acid	Yes 1 violation	No 1 violation	No 1 violation	No 1 violation	No 1 violation	0.85
α-Pinene	Yes 1 violation	No 1 violation	Yes	Yes	No 2 violations	0.55
Gallic acid	Yes 0 violations	No 2 violations	Yes	Yes	No 1 violation	0.56
Losartan	Yes 0 violations	Yes	Yes	Yes	Yes	0.56
Enalapril	Yes 0 violations	Yes	No 1 violation	Yes	Yes	0.55

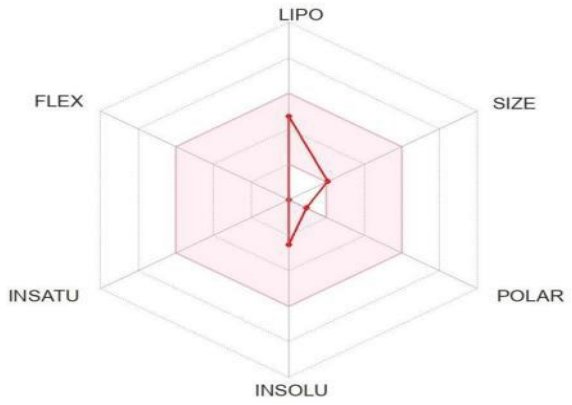
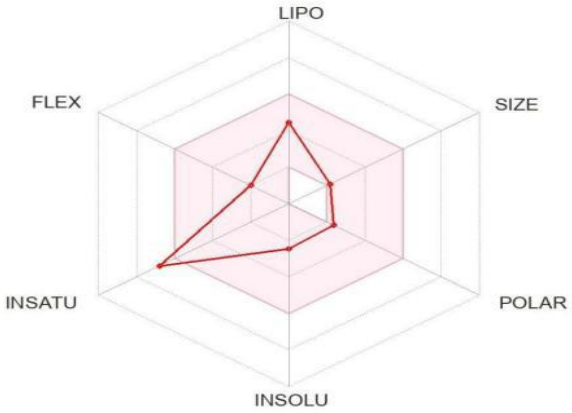
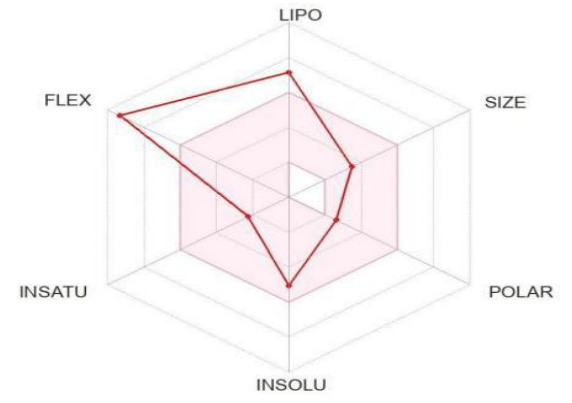
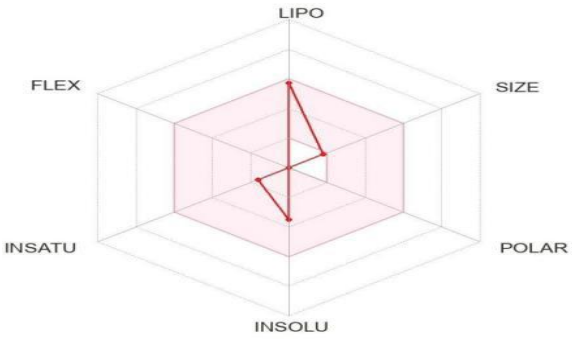
Drug-likeness assessment based on Lipinski, Ghose, Veber, Egan, and Muegge filters indicated that most compounds complied with accepted drug-likeness criteria. Berberine, Cubebin, Losartan, and Enalapril satisfied all major rules without significant violations. Linoleic acid exhibited a bioavailability score of 0.85 despite minor rule violations, while most other compounds demonstrated bioavailability scores of approximately 0.55, indicating acceptable oral drug potential.

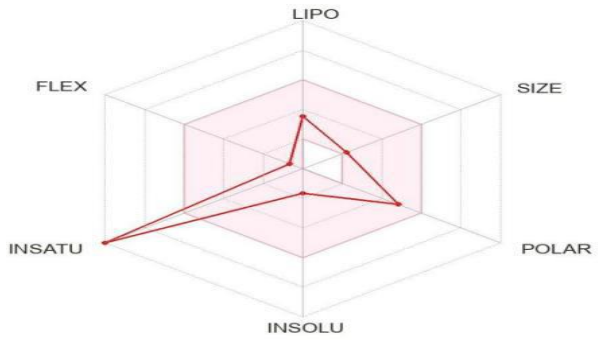
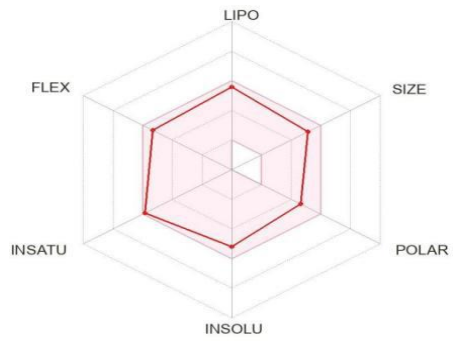
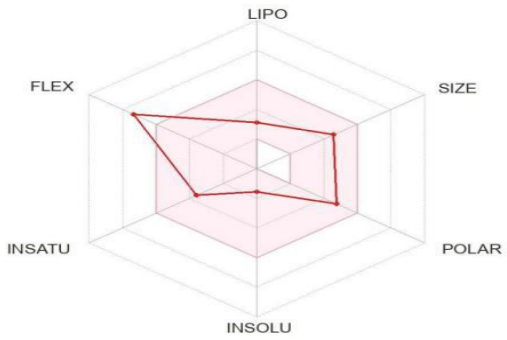
The present computational study demonstrated that the phytoconstituents of Sanjeevi Choornam possess promising nephroprotective activity against proteinuria through significant interactions with Nephrin and ACE. Among the compounds investigated, Cubebin emerged as the most promising lead molecule due to its superior binding affinity against ACE (−10.6 kcal/mol), exceeding that of the standard drug Enalapril. Macelignan and Berberine also exhibited strong binding interactions comparable to Losartan and demonstrated favorable pharmacokinetic properties. These findings suggest that the therapeutic efficacy of Sanjeevi Choornam may result from the synergistic action of multiple bioactive compounds targeting key molecular pathways involved in proteinuria and renal dysfunction. Further in vitro, in vivo, and clinical studies are warranted to validate these computational findings and establish the efficacy and safety of the formulation.

Table 9: ADME properties of the compounds.

Compound	ADME
Berberin	

Myristicin	
Macelignan	
Cubebin	

1,8-Cineole	
Eugenol	
Linoleic acid	
α-Pinene	

Gallic acid	
Losartan	
Enalapril	

CONCLUSION

The present in silico study demonstrated that the phytoconstituents of Sanjeevi Choornam possess promising nephroprotective potential against proteinuria through significant interactions with the key target proteins Nephrin and ACE. Molecular docking analysis revealed that Cubebin exhibited the highest binding affinity towards ACE (−10.6 kcal/mol), surpassing the standard drug Enalapril (−8.5 kcal/mol), while Macelignan and Berberine showed strong interactions with Nephrin comparable to the standard drug Losartan. These findings suggest that the selected phytocompounds may contribute to the preservation of glomerular filtration barrier integrity and modulation of renin–angiotensin system-mediated renal injury.

Furthermore, ADME analysis demonstrated favorable pharmacokinetic characteristics, including good gastrointestinal absorption, acceptable bioavailability, and satisfactory drug-likeness properties for most of the investigated compounds. The combined docking and ADME results indicate that the therapeutic efficacy of Sanjeevi Choornam may be attributed to the synergistic action of its bioactive constituents against multiple molecular pathways involved in proteinuria.

Overall, this study provides scientific evidence supporting the traditional Siddha use of Sanjeevi Choornam in the management of Mega Neer (urinary and renal disorders). However, further in vitro, in vivo, and clinical investigations are essential to validate the nephroprotective efficacy, safety, and therapeutic applicability of the formulation in proteinuria and chronic kidney disease.

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