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In Silico computational screening of *Amurthathi Chooranam* - Siddha Poly herbal formulation for management of Urolithiasis against target Tamm – Horsfall protein

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ABSTRACT

Urolithiasis plagues are common urinary disease in worldwide. Siddha medicine is one of the traditional systems of medicines practiced in the southern India. *Amurthathi Chooranam* is a classical Siddha Poly herbal formulation which was analyzed by molecular docking for Urolithiasis. The present study is aimed to accomplish the In Silico computational binding of phytocomponents of *Amurthathi Chooranam* with the core amino acids (CYS 527, PRO 528, HIS 529, GLY 534, ARG 583, THR 585, ARG 586) of the target protein Tamm–Horsfall protein (PDB) - 4WRN which is involved in calcium oxalate crystallization for management of urolithiasis. Docking calculations were carried out for retrieved phytocomponents against target protein Tamm–Horsfall protein. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Initial position, orientation and torsions of the ligand molecules were set randomly.Total 9 bioactive lead compounds were retrieved from the herbs present in *Amurthathi Chooranam* such as Alpha-phellandrene, Elemene, Elemicin, Tinosporide, Nerolidol, Eugenol, Quercetin, Syringic Acid and Morphine possess maximum of 3-4 interactions with the core active amino acid residues present on the target protein Tamm–Horsfall protein. Based on the results it was concluded that the above mentioned compounds exerts anti-urolithiasis activity by preventing calcium oxalate crystallization which inhibit the target Tamm–Horsfall protein for management of urolithiasis.

Keywords: Molecular Docking, *Amurthathi Chooranam*, Antiurolithiatic activity, Tamm–Horsfall protein

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INTRODUCTION

Urolithiasis is a global widespread disease with an increasing incidence over the recent decades and it is a major cause of morbidity and affects approximately 1–15% of the world's population. [1] The types of kidney stone include Calcium oxalate, Calcium phosphate, Uric acid, Struvite and mixed stones, among which calcium stones are the most common and include about 70 to 80% of the stones. [2]

Siddha Medicines refers Urolithiasis as *Kalladaippu*. According to *Yoogi Vaithiya Sindhamani* [3], *Kalladaippu* is classified into 4 types which follow:

1. Vali kalladaippu
2. Azhal kalladaippu
3. Aiya kalladaippu
4. Mukkutra kalladaippu

Many medicines common to all the four types of *Kalladaippu* have been prescribed in classical Siddha text books. *Amurthathi Chooranam* is one among the Siddha polyherbal formulation mentioned in *Anubava Vaithiya Deva Ragasiyam* [4] which is indicated for renal stones.

Even though several Siddha medicines are being used in the treatment of renal stones, most of the medicines have not been characterized by using modern scientific methods and the key bioactive components are yet to be explored to a greater extent. Standardization of Siddha herbal formulations is necessary to assess the quality of drugs for treatment processes.

Molecular docking is one of the in Silico methods which are the most efficient method when compared to in-vitro and in-vivo to find the active compound present in the medicinal plants. A three dimensional structure becomes very important in the molecular docking methods that shows the phyto compounds. During these present scenario advancements the study and documentation of structural compounds from medicinal plants are most important.[5] Utilization of computers and software are leading to the increased computing capabilities that provide opportunities to develop simulations and calculations in drug designing. This method includes a structure based drug design and ligand based drug design. In the field of structure based drug design molecular docking is commonly used to predict and inter molecular complex between the drug molecules with its target protein. [6]

Tamm–Horsfall (THP) is one of the main components of urinary protein. It is a glyco-protein produced and secreted by the thick ascendant limb of the loop of Henle, being the abundant protein in normal human urine; excreted in quantities of 20–200 mg/24 h. [7] THP of normal subjects inhibits the aggregation but has little effect on nucleation and growth of CaOx crystals. [8] Moreover, THP isolated from the urine of recurrent stone formers which removes it from effective interaction with CaOx monohydrate crystals. [9]

In modern drug designing, molecular docking is routinely used for understanding drug information about drug receptor interactions and is frequently used to predict the binding orientation of small molecules of drug to their targets protein in order to predict the affinity and activity of the small molecule.[10] The current paper deals with the 9 bioactive phytocomponents of *Amurthathi Chooranam* intracted with core amino acid by molecular docking to assess its antiurolithiatic property with target THP.

Aim and Objective

The present study is aimed to accomplish the In Silico computational binding of phytocomponents of *Amurthathi Chooranam* (*Anubava Vaithiya Deva Ragasiyam*) with the core amino acids (CYS 527, PRO 528, HIS 529, GLY 534, ARG 583, THR 585, ARG 586) of the targets by forming hydrogen bond will hinder the function of the target protein Tamm–Horsfall protein (PDB) - 4WRN which is involved in calcium oxalate crystallization. Thereby phytocomponents which inhibit the target Tamm–Horsfall protein may act as a potential therapeutic agent for management of urolithiasis and related symptoms.

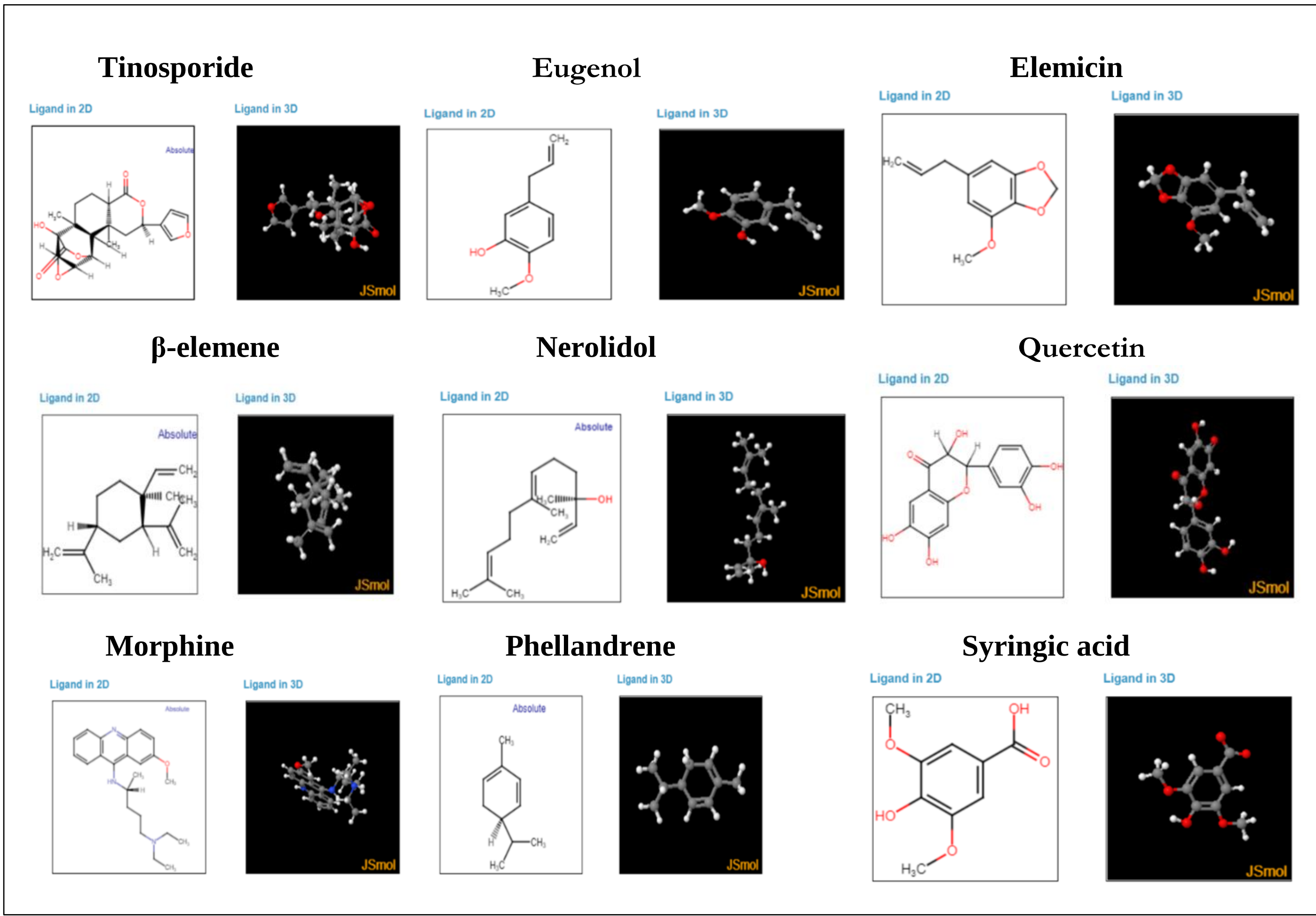
MATERIALS AND METHOD

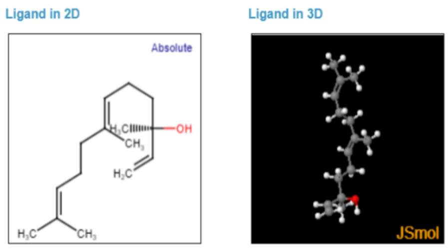
Lead Molecules from *Amurthathi Chooranam*

Docking calculations were carried out for the compounds retrieved from the herbal sources such as Alpha-phellandrene, Elemene, Elemicin, Tinosporide, Nerolidol, Eugenol, Quercetin, Syringic Acid and Morphine possess with the core active amino acid residues present on the target protein Tamm–Horsfall protein. (Table.1 & Fig A). The ligand molecular properties are illustrated in Table. 2

Table 1: List of Phytocomponents Selected for docking

Herb	**Phytochemicals**	**References**
Tinospora cordifolia	Tinosporide	[11]
Myristica fragrans	Eugenol Elemicin	[12]
Piper Cubeba	β-elemene	[13]
Eletaria cardamom	Nerolidol	[14]
Syzygium aromaticum	Quercetin	[15]
Papaver somniferum	Morphine	[16]
Taxus Baccata L	α- Phellandrene	[17]
Quercus infectoria oliver	Syringic acid	[18]





Quercetin

Figure A. 2D and 3D Structure of Phytocomponents

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
Tinosporide	374.4 g/mol	$C_{20}H_{22}O_7$	1	7	1
Eugenol	164.2 g/mol	$C_{10}H_{12}O_2$	1	2	3
Elemicin	208.25 g/mol	$C_{12}H_{16}O_3$	0	3	5
β-elemene	204.35 g/mol	$C_{15}H_{24}$	0	0	3
Nerolidol	222.37 g/mol	$C_{15}H_{26}O$	1	1	7
Quercetin	302.23 g/mol	$C_{15}H_{10}O_7$	5	7	1
Morphine	285.34 g/mol	$C_{17}H_{19}NO_3$	2	4	0
α- Phellandrene	136.23 g/mol	$C_{10}H_{16}$	0	0	1
Syringic acid	198.17 g/mol	$C_9H_{10}O_5$	2	5	3

TARGET DETAILS AND RECEPTOR STRUCTURE

Crystalline structure of the target protein Tamm–Horsfall protein (PDB) - 4WRN was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

PDB	Name of the Target
4WRN	Tamm–Horsfall protein

3D- Structure of Tamm–Horsfall protein (PDB) - 4WRN

Figure: b. Receptor structure

TOOL FOR STUDY [19, 20, 21, 22]

Docking calculations were carried out for retrieved phytocomponents against target protein Tamm–Horsfall protein. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (*Morris, Goodsell et al., 1998*). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell et al., 1998*). 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 and Wets, 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.

RESULTS AND DISCUSSION

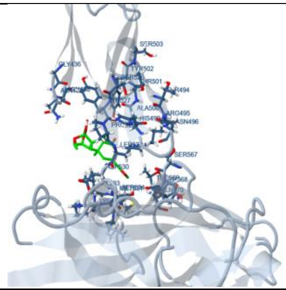
Total of 9 bioactive lead compounds were retrieved from the herbs present in the Siddha formulation ***Amurthathi Chooranam***. From reported data of the herb, the phytochemicals such as Elemene, Elemicin, Tinosporide, Nerolidol, Eugenol, Quercetin, Syringic Acid and Morphine possess maximum of 3-4 interactions with the core active amino acid residues present on the target protein Tamm–Horsfall protein. The interaction brings an idea that these phytochemicals may inhibit calcium oxalate crystallization in urolithiatic condition. Summary of the molecular docking studies of the lead compounds against Tamm–Horsfall protein, Amino acid Residue Interaction have been tabulated Table 3 & 4 and docking pose in Figure C

Table: 3. Summary of the molecular docking studies of compounds against Tamm–Horsfall protein (PDB) - 4WRN

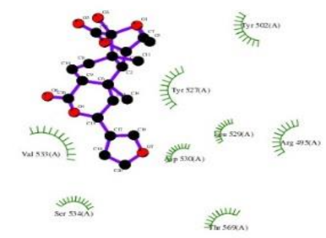
Compound	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Tinosporide	-3.80 kcal/mol	1.63 Mm	-0.02 kcal/mol	-4.64 kcal/mol	481.297
Eugenol	-4.14 kcal/mol	930.83 Um	-0.01 kcal/mol	-4.61 kcal/mol	452.908
Elemicin	-4.48 kcal/mol	521.46 Um	-0.01 kcal/mol	-5.44 kcal/mol	525.883
β-elemene	-3.80 kcal/mol	1.63 mM	-0.02 kcal/mol	-4.64 kcal/mol	481.297
Nerolidol	-5.12 kcal/mol	177.98 Um	-0.03 kcal/mol	-7.38 kcal/mol	621.889
Quercetin	-5.79 kcal/mol	56.71 Um	-0.34 kcal/mol	-5.03 kcal/mol	501.054
Morphine	-5.03 kcal/mol	205.36 uM	-1.36 kcal/mol	-8.55 kcal/mol	682.187
α-Phellandrene	-4.17 kcal/mol	873.87 Um	-0.02 kcal/mol	-4.47 kcal/mol	427.504
Syringic acid	-3.66 kcal/mol	2.09 Mm	-0.18 kcal/mol	-4.20 kcal/mol	450.338

Table: 4. Amino acid Residue Interaction of Lead and Standard against Tamm–Horsfall protein (PDB) - 4WRN

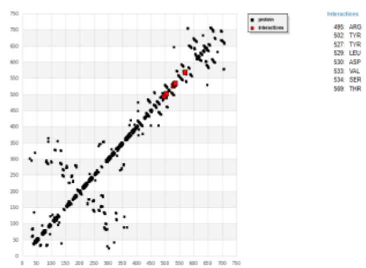
Compounds	Interactions	Amino acid Residues									
Alpha-phellandrene	1	498 THR	499 HIS	528 PRO	639 GLU	643 SER	644 SER	645 GLN	647 ARG		
Elemene	3	495 ARG	500 ALA	502 TYR	527 TYR	528 PRO	529 LEU				
Elemicin	3	500 ALA	502 TYR	527 TYR	529 LEU	530 ASP	533 VAL	534 SER	569 THR		
Tinosporide	3	495 ARG	500 ALA	527 TYR	529 LEU	530 ASP	533 VAL	534 SER	569 THR		
Nerolidol	4	495 ARG	500 ALA	502 TYR	527 TYR	528 PRO	529 LEU	533 VAL	534 SER	537 THR	569 THR
Eugenol	3	495 ARG	500 ALA	502 TYR	527 TYR	529 LEU	533 VAL	534 SER	569 THR		
Quercetin	3	438 ASN	500 ALA	502 TYR	527 TYR	528 PRO	529 LEU				
Syringic Acid	3	495 ARG	500 ALA	502 TYR	527 TYR	529 LEU	530 ASP	533 VAL	534 SER		
Morphine	3	495 ARG	502 TYR	527 TYR	528 PRO	529 LEU	530 ASP	533 VAL	645 GLN		



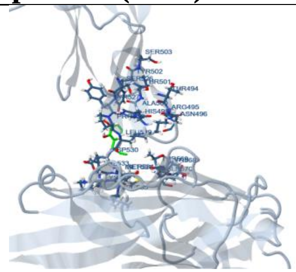
Tinosporide with Tamm–Horsfall protein (PDB) - 4WRN



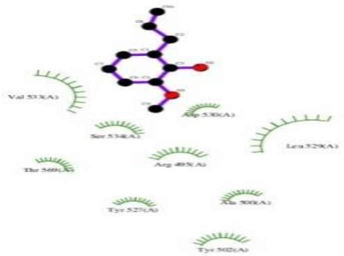
2D Interaction Plot Analysis



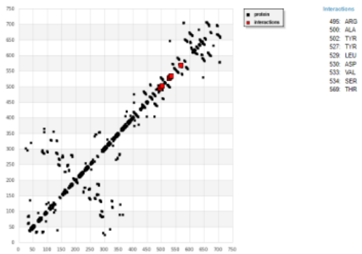
Hydrogen bond plotting with core amino acid Analysis



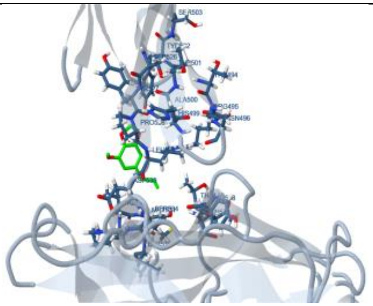
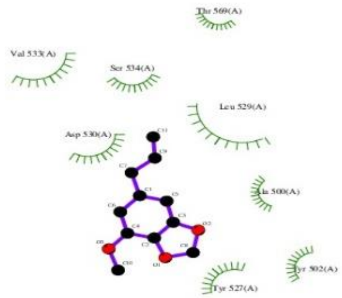
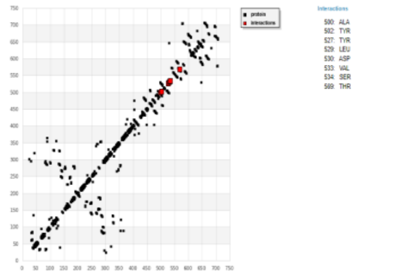
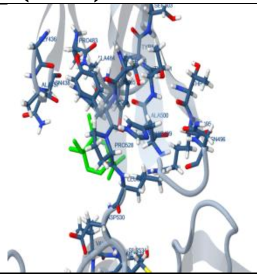
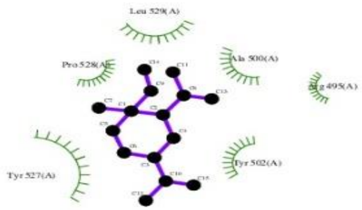
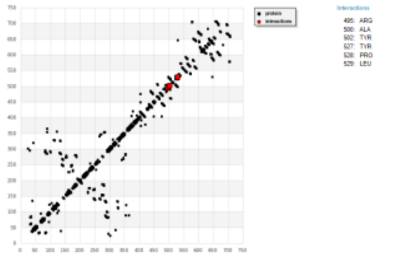
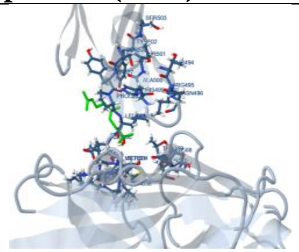
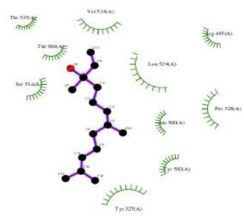
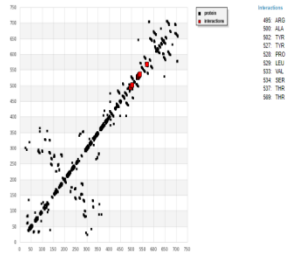
Eugenol with Tamm–Horsfall protein (PDB) - 4WRN

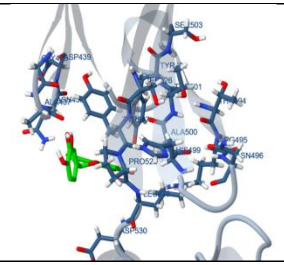
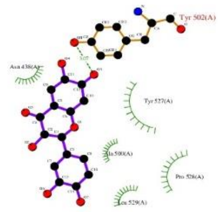
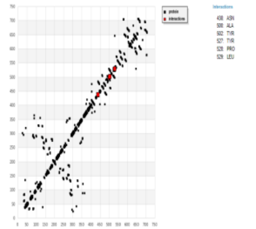
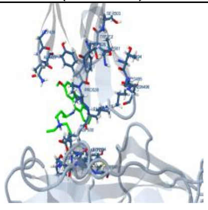
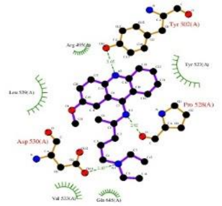
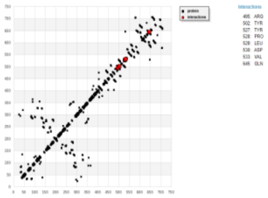
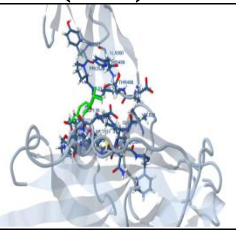
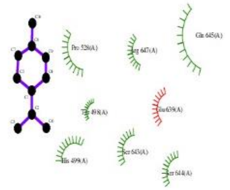
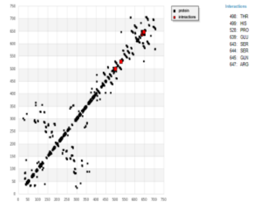
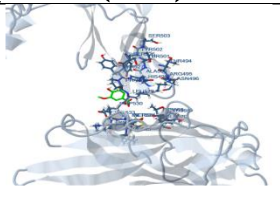
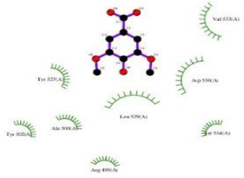
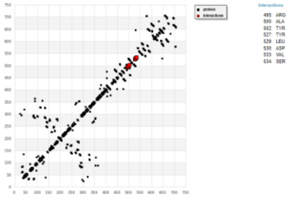


2D Interaction Plot Analysis



Hydrogen bond plotting with core amino acid Analysis

		
Elemicin with Tamm–Horsfall protein (PDB)- 4WRN 2D	**Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**
		
β-elemene with Tamm–Horsfall protein (PDB) 4WRN	**2D Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**
		
Nerolidol with Tamm–Horsfall protein (PDB) –4WRN 2D	**Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**

		
Quercetin with Tamm–Horsfall protein (PDB)- 4WRN	**2D Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**
		
Morphine with Tamm–Horsfall protein (PDB) 4WRN	**2D Interaction Plot Analysis**	**Hydrogen bond plotting with-core amino acid Analysis**
		
Phellandrene with Tamm–Horsfall protein (PDB) 4WRN	**2D Interaction Plot Analysis**	**Hydrogen bond plotting Analysis with core amino acid**
		
Syringic acid with Tamm–Horsfall protein (PDB) 4WRN	**2D Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**

CONCLUSION

For pharmacological validation of *Amurthathi Choornam*, the docking study was an important preliminary step for its scientific justification. Based on the results of the computational analysis it was concluded that the bioactive compound's like Elemene, Elemicin, Tinosporide, Nerolidol, Eugenol, Quercetin, Syringic Acid and Morphine present in the herbal ingredients reveals significant binding against the target Tamm–Horsfall protein by interacting with active amino acid present on the active site thereby it was concluded that these compounds may exerts anti-urolithiasis activity by preventing Calcium Oxalate crystallization. Thereby phytocomponents which inhibit the target Tamm–Horsfall protein may act as a potential therapeutic agent for management of urolithiasis. It was concluded that the phytochemicals present in the Siddha formulation ***Amurthathi Chooranam*** reveals significant anti- urolithiasis activity (Particularly Calcium Oxalate type of stone).

AUTHOR CONTRIBUTIONS

Dr. Ramani Mani structured and prepared the MS draft. *Prof. Dr. Kabilan Natarajan* provided useful inputs, modulated and corrected the MS draft. *Prof. Dr. Kanakavalli Kadarkarai* edited and finalized the review. All authors checked the final MS draft.

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CONFLICTS OF INTEREST

The author has no conflicts of interest to share.

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