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Anti-Urolithiasis Effect of *Siddha* Formulation *Sagalanoi chooranam* Against Tamm–Horsfall Protein using *In Silico* Model

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Abstract: *Siddha* is one of the most ancient medical system of India and as the treatment of choice for various diseases. Urolithiasis is one of the major diseases of the urinary tract and is a major source of morbidity and its re-occurrence rate is 70-81% in males and 47-60% in females. The objective of the present study was to perform the *in silico* computational studies of *Sagalanoi Chooranam* and to determine its efficacy in treatment and management of Urolithiasis. Auto dock program was used for the molecular docking studies against Tamm–Horsfall protein. The reported data of the phytochemicals such as Thiamine, Carvacrol, Cuminaldehyde, Glabridin, Glycyrrhizin, Oleic acid, Limonene and Epicatechin possess maximum of 2-3 interactions with the core active amino acid residues present on the target protein Tamm–Horsfall protein and helps in treatment and management of urolithiasis. These phytocomponents which inhibit the target Tamm–Horsfall protein may act as a potential therapeutic agent for management of urolithiasis. It is concluded that the phytochemicals present in the siddha formulation reveals significant lithotriptic activity.

Keywords: *Siddha* medicine, Tamm–Horsfall protein, Urolithiasis, Docking study, *Sagalanoi chooranam,* Lithotriptic

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Introduction

Urolithiasis is most common disease in the urinary tract. It occurs in approximately 12% of the global population and its re-occurrence rate is 70-81% in males and 47-60% in females. It is evaluated that at least 10% of the people in the world are suffering with the problem of urolithiasis. The Southern part has less occurrence of renal calculi when compared with other parts. The rate of occurrence is three times higher in men than in women. It is because the calculi formation is enhanced by testosterone and inhibited by oestrogen. The most common types of renal calculi are struvite (magnesium ammonium phosphate), calcium oxalate, urate, cysteine and silica (Vijaya

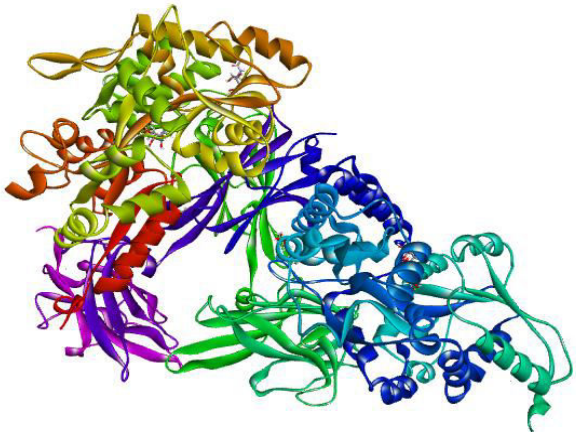

Fig. 1: 3D- Structure of Tamm–Horsfall protein (PDB) - 4WRN.

et al., 2013). The particularly macrophage differentiation in immune response and play crucial roles in renal calcium oxalate crystal formation (Wang *et al.*, 2021).

The most frequent component of calculi is calcium oxalate (75%–90%), followed by uric acid (5%–20%), calciphosphate (6%–13%), struvite (2%–15%), apatite (1%) and cystine (0.5%–1%). The prevalence of Urolithiasis is only 1%–8% in most part of East Asia and North Asia. The incidence of urolithiasis reaches its peak in population aged over 30 years (Liu *et al.*, 2018).

Tamm-Horsfall (TH) glycoprotein is a major protein present in normal urine and is the primary component of waxy nephron casts. It is of renal origin and localized in the distal convoluted tubule and in the thick ascending limb of the loop of Henle. It is characterized by its tendency to form aggregates in the presence of enhance concentrations of NaCl or H^+(Peraldi, 1992) .

The siddha formulation *Sagalanoi Chooranam* consist of *Coriandrum sativum*, *Cuminum cyminum*, *Glycyrrhiza glabra*, *Nigella sativa*, *Cyas circinalis*. The phytochemical present in *Corandrum sativum* is Thiamine (Ali, 2016). *Cuminum cyminum* contains Carvacrol, cuminaldehyde, coumaric acid (Pastorino *et al.*, 2018). *Glycyrrhiza glabra* contains glabridin, glycyrrhizin (Yimer *et al.*, 2019). *Nigella sativa* contains oleic acid, limonene (Abeer, 2010). *Cyas circinalis* contains epicatechin (Bikadi and Hazai, 2009). The phytocomponents inhibit the target Tamm–Horsfall protein and may act as a potential therapeutic agent for management of urolithiasis and related symptoms.

The main objective of the present study was to find the binding ability of phytocomponents with the core amino acids (527, 528, 529, 534, 583, 585, 586) of the targets which will hinder the function of the target protein Tamm–Horsfall protein (PDB) - 4WRN involved in calcium oxalate crystallization and find its ability in acting as a potential therapeutic agent for management of urolithiasis and related symptoms.

Materials and Methods

Binding ability of phytocomponents with the core amino acids (527, 528, 529, 534, 583, 585, 586) of the targets by forming hydrogen bond which is involved in calcium oxalate crystallization which will hinder the function of the target protein Tamm–Horsfall protein (PDB) - 4WRN.

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 Auto dock program and the best dock pose was selected based on the interaction study

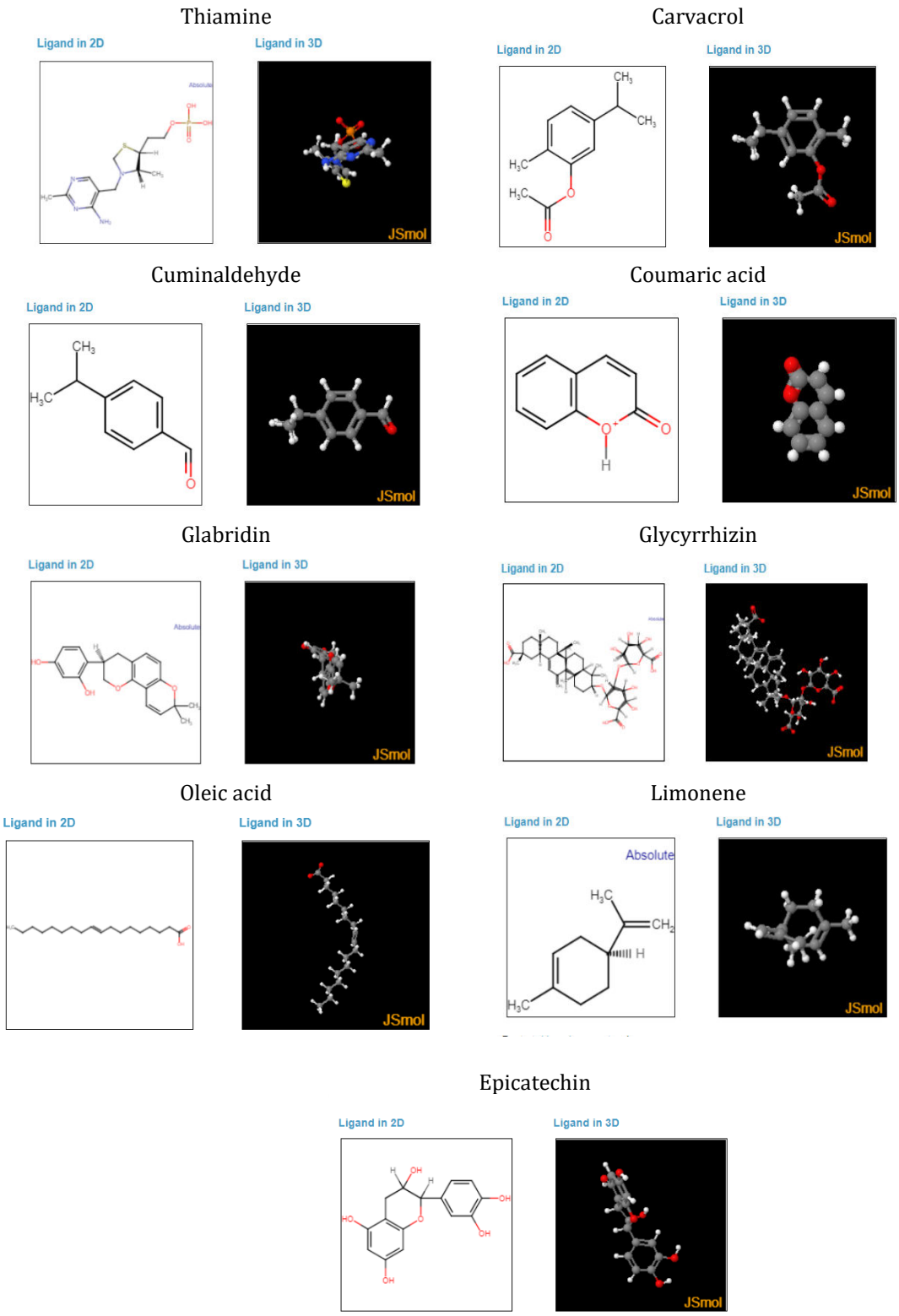

Fig. 2: 2D and 3D Structure of Phytocomponents.

Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Thiamine	265.36 g/mol	$C_{12}H_{17}N_4OS^+$	2	5	4
Carvacrol	150.221 g/mol	$C_{10}H_{14}O$	1	1	1
Cuminaldehyde	148.205 g/mol	$C_{10}H_{12}O$	0	1	2
Coumaric acid	164.16 g/mol	$C_9H_8O_3$	2	3	2
Glabridin	324.4 g/mol	$C_{20}H_{20}O_4$	2	4	1
Glycyrrhizin	822.9 g/mol	$C_{42}H_{62}O_{16}$	8	16	7
Oleic acid	282.5 g/mol	$C_{18}H_{34}O_2$	1	2	15
Limonene	136.23 g/mol	$C_{10}H_{16}$	0	0	1
Epicatechin	290.271 g/mol	$C_{15}H_{14}O_6$	5	6	1

Table 2: 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
Thiamine	-7.80 kcal/mol	1.91 uM	-0.34 kcal/mol	-6.02 kcal/mol	592.398
Carvacrol	-4.19 kcal/mol	845.89 uM	-0.11 kcal/mol	-5.07 kcal/mol	501.383
Cuminaldehyde	-3.90 kcal/mol	1.38 mM	-0.02 kcal/mol	-4.50 kcal/mol	435.514
Coumaric acid	-3.94 kcal/mol	1.30 mM	-0.14 kcal/mol	-3.94 kcal/mol	386.476
Glabridin	-6.32 kcal/mol	23.39 uM	-0.18 kcal/mol	-7.22 kcal/mol	657.481
Glycyrrhizin	-6.35 kcal/mol	22.20 uM	-1.04 kcal/mol	-5.85 kcal/mol	1015.199
Oleic acid	-3.38 kcal/mol	3.31 mM	-0.06 kcal/mol	-3.68 kcal/mol	329.543
Limonene	-3.94 kcal/mol	1.30 mM	-0.09 kcal/mol	-4.24 kcal/mol	426.701
Epicatechin	-5.52 kcal/mol	89.83 uM	-0.16 kcal/mol	-5.59 kcal/mol	606.828

analysis.

Docking Methodology:

Target protein Tamm–Horsfall protein against Docking calculations were carried out for retrieved phytocomponents. Kollman united atom type charges, Essential hydrogen atoms, and solvation parameters were added with the aid of AutoDock tools (Morris *et al.,* 1999). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (Morris *et al.,* 1999). AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. The Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method (Solis and Wets, 1981) using Docking simulations were performed. Randomly set were Initial position, orientation, and torsions of the ligand molecules. All rotatable torsions were

Table 3: Amino acid Residue Interaction of Lead and Standard against Tamm–Horsfall protein (PDB) - 4WRN

Compounds	Interactions	Amino acid Residues									
Thiamine	3	495 ARG	500 ALA	502 TYR	527 TYR	529 LEU	530 ASP	533 VAL	534 SER	569 THR	
Carvacrol	2	484 ALA	495 ARG	502 TYR	527 TYR	529 LEU					
Cuminaldehyde	3	495 ARG	500 ALA	527 TYR	529 LEU	534 SER	569 THR				
Coumaric acid	1	498 THR	528 PRO	530 ASP	639 GLU	643 SER	644 SER	647 ARG			
Glabridin	3	495 ARG	500 ALA	502 TYR	527 TYR	528 PRO	529 LEU				
Glycyrrhizin	2	438 ASN	484 ALA	502 TYR	527 TYR	528 PRO	530 ASP	533 VAL	643 SER	644 SER	
Oleic acid	2	437 ALA	484 ALA	500 ALA	502 TYR	527 TYR	529 LEU	530 ASP	533 VAL	569 THR	
Limonene	2	495 ARG	500 ALA	527 TYR	529 LEU						
Epicatechin	3	495 ARG	502 TYR	527 TYR	529 LEU	533 VAL	534 SER	569 THR			

released during docking. From 2 different runs that were set to terminate after a maximum of 250000 energy evaluations that Each docking experiment was derived. 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 (Solis and Wets, 1981; Halgren, 1998; Morris *et al.*, 1999).

Results and Discussion

Tamm-Horsfall (TH) glycoprotein is a major protein present in normal urine and is the primary component of waxy nephron casts. This protein is of renal origin and has been localized in the thick ascending limb of the loop of Henle and in the distal convoluted tubule. Its physicochemical properties are characterized by its tendency to form aggregates in the presence of increasing concentrations of NaCl or H^+. The primary structure of Tamm-Horsfall protein has been characterized and was found to be similar to uromodulin, a glycoprotein purified from the urine of pregnant women having immunosuppressive properties. TH protein has a different glycosy-lation since it does not have the same immunosuppressive properties as uromodulin. TH protein is anchored to the cytoplasmic membrane via a phosphatidyl-inositol group and could be secreted in urine after cleavage by a specific phospholipase. The role of TH protein in physiological and pathological states, its regulation are still unknown (Önder, 2018).The molecular docking studies were carried out for Thiamine, Carvacrol, Cuminaldehyde, Coumaric acid, Glabridin, Glycyrrhizin, Oleic acid, Limonene and Epicatechin present in ingredients of *Sagalanoi Chooranam* against Tamm–Horsfall protein to identify the molecular interactions.

A total of 9 bioactive lead compounds were obtained from the herbs in accordance with the literature. On comparing binding affinities of the compounds ,it was found that Thiamine showed the hightest binding affinity of -7.80 kcal/mol. Glycyrrhizin and Glabridin showed the second-hightest binding affinity with the binding free energy of -6.35 Kcal/mol and -6.32 Kcal/mol. Followed by Epicatechin had -5.52 kcal/mol, Carvacrol had -4.19kcal/mol,Coumaric acid and Limonene had -3.94kcal/mol,Cuminaldehyde had -3.90kcal/mol,Oleic acid had -3.38kcal/mol. So the compounds Thiamine, Cuminaldehyde Glabridin and Epicatechin of *Sagalanoi Chooranam* possess

100% binding efficacy by interacting with all three-core target amino acid (534 ASP, 529 ASP, 527ASP) present on target receptor Tamm–Horsfall protein. Hence it helps in treatment and management of Urolithiasis.

Conclusion

Based on the results of the computational analysis it is concluded that the bio-active compound's like Thiamine, Carvacrol, Cuminaldehyde, Glabridin, Glycyrrhizin, Oleic acid, Limonene and Epicatechin present in the herbal ingredients revels 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 promising 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 reveals significant anti- urolithiasis activity.

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References

Abeer M. (2010) Phytochemical investigation of *Cycas circinalis* and *Cycas revoluta* leaflets: Moderately active antibacterial biflavonoids. Planta Med. 76(8): 796-802.

Ali EAS. (2016) The pharmacological activities of Cuminum cyminum - A review. IOSR J Pharmacy 6(6): 46-65.

Bikadi Z, Hazai E. (2009) Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminf. 1: 15.

Halgren TA. (1998) Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94. J Computational Chem. 17(5-6): 490-519.

Liu Y, Chen Y, Liao B, Luo D, Wang K, Li H and Zeng G. (2018) Epidemiology of urolithiasis in Asia. Asian J Urol. 5(4): 205-214.

Morris GM, David SG, Robert SH, Ruth H, William EH, Richard KB and Arthur JO. (1999) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Computational Chem. 19(14), 1639-1662.

Önder A. (2018) Coriander and its phytoconstituents for the beneficial effects. Potential of essential oils. InTech. doi:10.5772/intechopen.78656.

Pastorino G, Cornara L, Soares S, Rodrigues F and Oliveira MBPP. (2018) Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review. Phytother Res. 32(12): 2323-2339.

Peraldi MN. (1992) Le point sur la protéine de Tamm-Horsfall [Tamm-Horsfall protein]. Nephrologie 13(1): 7-11.

Solis FJ and Wets RJB. (1981) Minimization by random search techniques. Mathematics Operations Res. 6(1): 19-30.

Vijaya T, Kumar MS, Ramarao NV, Babu AN and Ramarao N. (2013) Urolithiasis and its causes-short review. J Phytopharmacol. 2(3): 1-6.

Wang Z, Zhang Y, Zhang J, Deng Q and Liang H. (2021) Recent advances on the mechanisms of kidney stone formation (Review). Int J Mol Med. 48(2):149.

Yimer EM, Tuem KB, Karim A, Ur-Rehman N and Anwar F. (2019) *Nigella sativa* L. (Black Cumin): A promising natural remedy for wide range of illnesses. Evid Based Complement Alternat Med. 2019: 1528635.