



**MOLECULAR DOCKING STUDIES OF SIDDHA HERBAL FORMULATION
KALLADAIPPU KUDINEER ON TAMM – HORSEFALL PROTEIN INVOLVED IN
CALCIUM OXALATE CRYSTALLIZATION OF UROLITHIASIS**

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Article Received on 12/08/2024

Article Revised on 02/09/2024

Article Accepted on 23/09/2024

ABSTRACT

Siddha medicine is one of the most ancient medical systems of India and is the mother medicine of ancient Tamils / Dravidians of peninsular south India. Fundamental principles of siddha include theories of five elements (Aimpotham) and three humors (mukkutram). According to classical siddha system of medicine, the clinical features of Kalladaippu noi is widely correlated with the disease called Urolithiasis (Renal stone), which is a common disorder found in humans of all ages due to changing lifestyle and dietary choices that leading to increased incidence and prevalence around the globe. In siddha medicinal literatures, there are many herbs which are used to prevent the recurrence of stone formation through anti-inflammatory, lithotriptic, anti-spasmodic action. This article reviews about the anti urolithiatic activity of herbal formulation *Kalladaippu kudineer* interaction with Tamm – Horsefall -Protein involve in ca. oxalate crystallization.

KEYWORDS: Urolithiasis, Anti urolithiatic activity, Tamm- Horsefall Protien.

INTRODUCTION

In the classical siddha literatures, Kalladippu comes under Neerinai Arukkal noigal, which means disease that reduces urinary output. According to traditional siddha text *Yugi vaithiya chinthamani*, the kalladaippu noi correlates with the modern condition called Urolithiasis. Kalladippu noi is classified into four types, that are Vali, Azhal, Iyam, Mukkutram by yugi muni based on three thodam. It is caused by intake of impure stagnant water, contaminated food and high content of carbohydrate foods and by controlling urine and semen. Urolithiasis is one of the most common distress of urinary tract. Calculus formation at any region in the urinary tract–kidney to urethra, lead to urolithiasis. It's clinical features are dysuria, burning micturition, blockage of urine flow and hydronephrosis. The symptoms are colicky pain in the loin that radiating to groin, nausea, vomiting, fever, pain in the genital regions and less frequently hematuria, diarrhoea and headache.

Urinary stones affected 10 -12% of the population in industrialized countries. The epidemiology of urolithiasis differs according to geographical area in terms of prevalence incidence, age, sex distribution, stone composition and stone location. Men commonly affected

than women to the ratio of 3:1. The peak age for developing stone is between 30 to 50 occurs more frequently. About 75% of renal stones are composed of either calcium oxalate or calcium oxalate crystals mixed with calcium phosphate.

The siddha medicinal texts describes certain drugs with lithotriptic and diuretic action that successfully treat urolithiasis. Kalladaippu kudineer is one of the herbal formation mentioned in the siddha text "*Agasthiyar Gunavagada Thirattu*". *Zingiber officinale*, *Aerva lanata*, *Tribulus terrestris*, *Crateva religiosa*, *Pavonia odorata* are the ingredients that contained in this herbal formulation.

For a broader research perspective, the lead molecules of this formulation were interacted on Tamm- Horsefall Protien with its anti- urolithiatic activity. This article may help to evaluate the efficiency of this Kalladaippu kudineer with its anti- urolithiatic property.

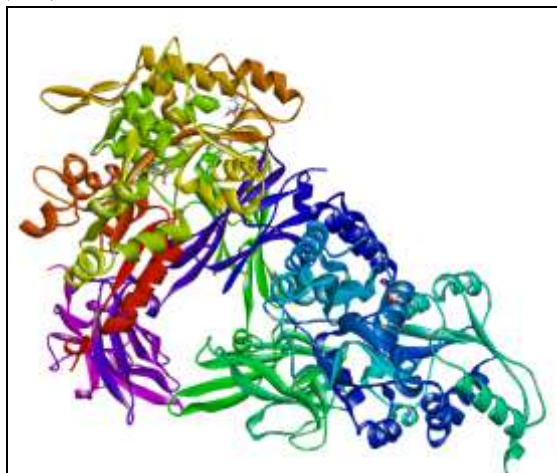
ANTI- UROLITHIASIS ACTIVITY**List of Phytochemicals Selected for docking**

Herbs	Phytochemicals	References
Zingiber Officinale	Gingerenone-A	Sahdeo Prasad. Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer. <i>Gastroenterology Research and Practice</i> , 2015; 1-11.
Aerva Lanata	Kaempferol, Quercetin	Goyal, M., Pareek, A., Nagori, B. P., & Sasmal, D. Aerva lanata: A review on phytochemistry and pharmacological aspects. <i>Pharmacognosy reviews</i> , 2011; 5(10): 195–198. https://doi.org/10.4103/0973-7847.91120
Tribulus Terrestris	Rutin β -sitosterol	Chhatre, S., Nesari, T., Somani, G., Kanchan, D., & Sathaye, S. Phytopharmacological overview of Tribulus terrestris. <i>Pharmacognosy reviews</i> , 2014; 8(15): 45–51. https://doi.org/10.4103/0973-7847.125530
Crateva Religiosa	β -caryophyllene	V.Srinivas. A Scientific review on Crateva religiosa. <i>IJAPBC</i> , 2018; 7(1): 11-16.
Pavonia Odorata	Palmitic acid	Biochemistry and Therapeutic Uses of Medicinal Plants Publisher: Discovery Publishing House Pvt. Ltd., New Delhi (India) Editors: Abbas Ali Mahdi, Y.K. Sharma, Murtaza Abid, M.M. Abid Ali Khan. 2017.

OBJECTIVE

Binding of phytochemicals 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 phytochemicals which inhibit the target Tamm–Horsfall protein may act as a potential therapeutic agent for management of urolithiasis and related symptoms.

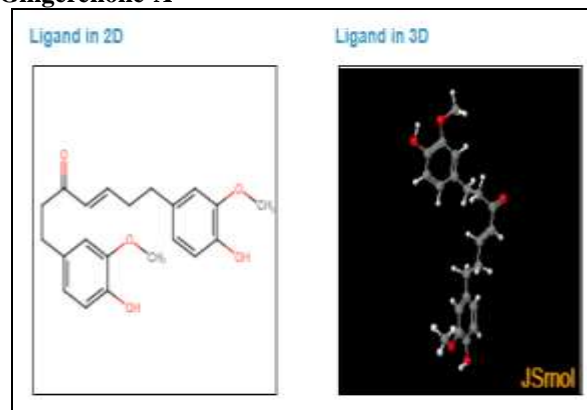
PDB	Name of the Target
4WRN	Tamm–Horsfall protein

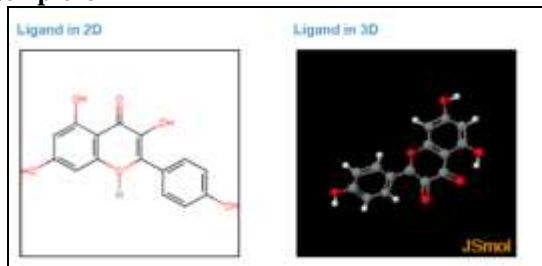
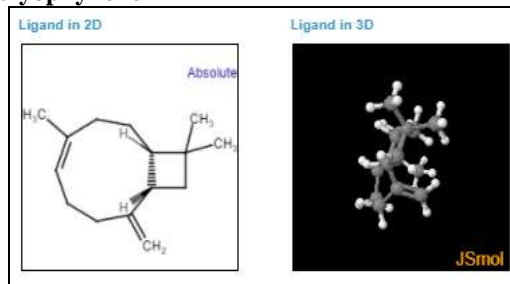
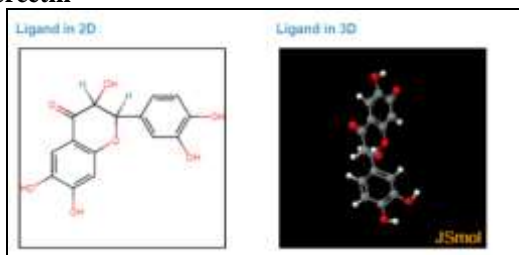
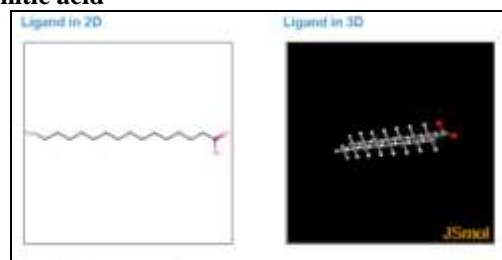
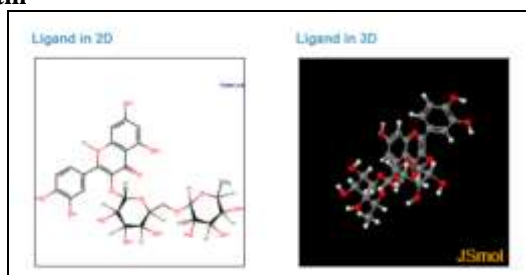
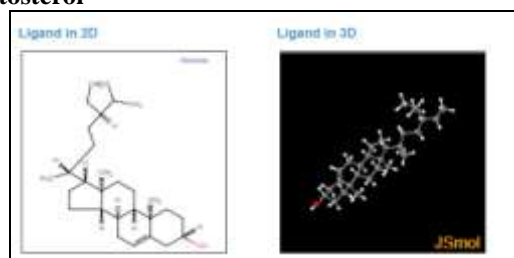
3D- Structure of Tamm–Horsfall protein (PDB) - 4WRN**RECEPTOR STRUCTURE**

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 Autodock program and the best dock pose was selected based on the interaction study analysis.

METHODOLOGY

Docking calculations were carried out for retrieved phytochemicals 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 $\times\times$ 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.

2D and 3D Structure of Phytochemicals Gingerenone-A

Kaempferol **β -caryophyllene****Quercetin****Palmitic acid****Rutin** **β -sitosterol****Ligand Properties of the Compounds Selected for Docking Analysis**

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Gingerenone-A	356.4 g/mol	$C_{21}H_{24}O_5$	2	5	9
Kaempferol	286.24 g/mol	$C_{15}H_{10}O_6$	4	6	1
Quercetin	302.23 g/mol	$C_{15}H_{10}O_7$	5	7	1
Rutin	610.5 g/mol	$C_{27}H_{30}O_{16}$	10	16	6
β -sitosterol	414.7g/mol	$C_{29}H_{50}O$	1	1	6
β -caryophyllene	204.35 g/mol	$C_{15}H_{24}$	0	0	0
Palmitic acid	256.42 g/mol	$C_{16}H_{32}O_2$	1	2	14

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

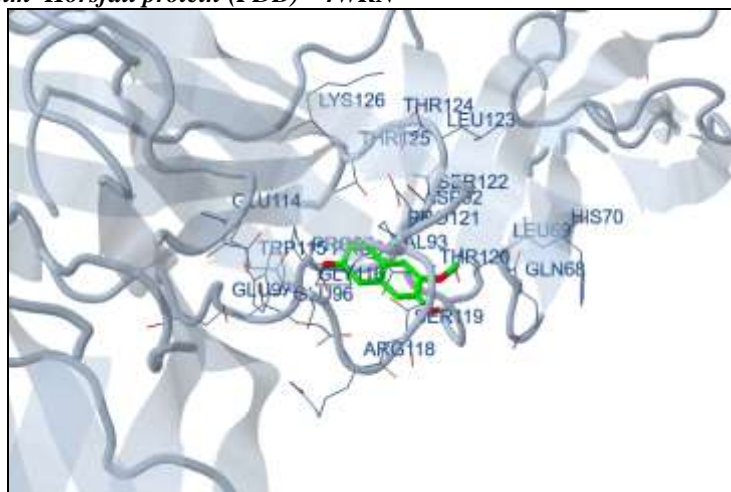
Compound	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Gingerenone-A	-7.34 kcal/mol	4.19 uM	-0.02 kcal/mol	-6.36 kcal/mol	562.298
Kaempferol	-4.99 kcal/mol	221.59 uM	-0.34 kcal/mol	-5.45 kcal/mol	555.923
Quercetin	-6.33 kcal/mol	22.83 uM	-0.60 kcal/mol	-5.67 kcal/mol	608.573
Rutin	-10.47 kcal/mol	21.20 nM	-0.21 kcal/mol	-5.60 kcal/mol	704.742
β -sitosterol	-8.06 kcal/mol	1.24 uM	-0.01 kcal/mol	-9.37 kcal/mol	760.733
β -caryophyllene	-5.28 kcal/mol	135.56 uM	-0.02 kcal/mol	-5.28 kcal/mol	507.24
Palmitic acid	-4.03 kcal/mol	1.11 mM	-0.40 kcal/mol	-7.57 kcal/mol	651.903

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

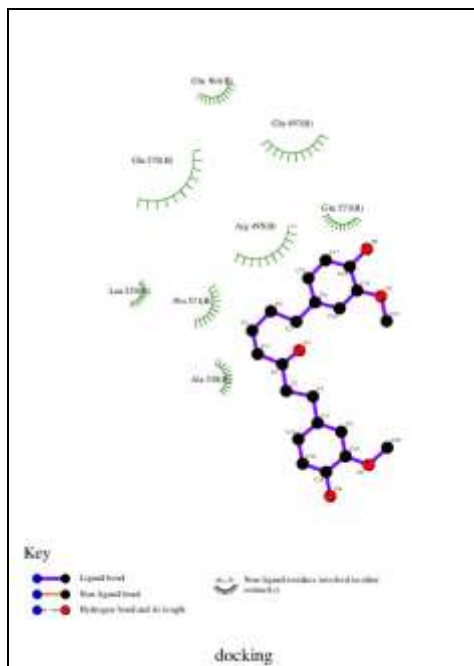
Compounds	Interactions	Amino acid Residues									
		495	497	529	538	564	570	571	573		
Gingerenone-A	1	ARG	GLU	LEU	ALA	GLN	GLN	PRO	GLN		
Kaempferol	4	ALA	TYR	TYR	PRO	LEU	ASP	VAL	SER		
Quercetin	3	438	484	495	502	527	529	530	534	569	
		ASN	ALA	ARG	TYR	TYR	LEU	ASP	SER	THR	
Rutin	1	528	530	639	642	643	644	645			
		PRO	ASP	GLU	GLU	SER	SER	GLN			
β -sitosterol	2	437	484	500	502	527	528	530	643	644	645
		ALA	ALA	ALA	TYR	TYR	PRO	ASP	SER	SER	GLN
β -caryophyllene	2	484	495	500	502	527	529				
		ALA	ARG	ALA	TYR	TYR	LEU				
Palmitic acid	3	484	495	502	527	529	530	533	534	569	
		ALA	ARG	TYR	TYR	LEU	ASP	VAL	SER	THR	

Docking Pose

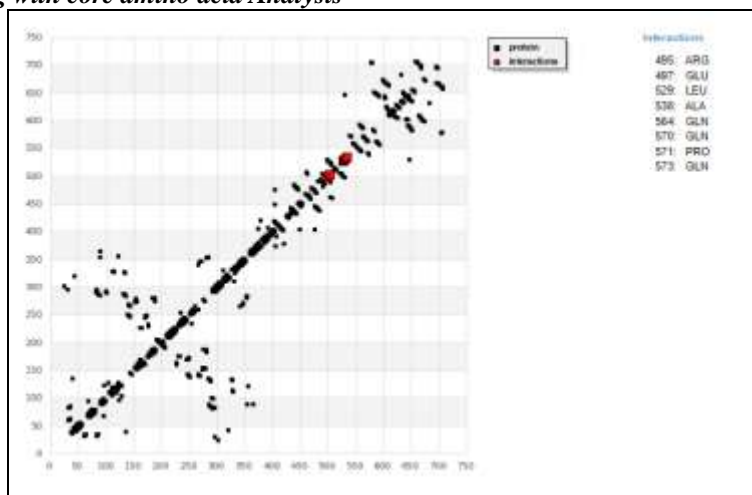
Gingerenone-A with Tamm–Horsfall protein (PDB) - 4WRN



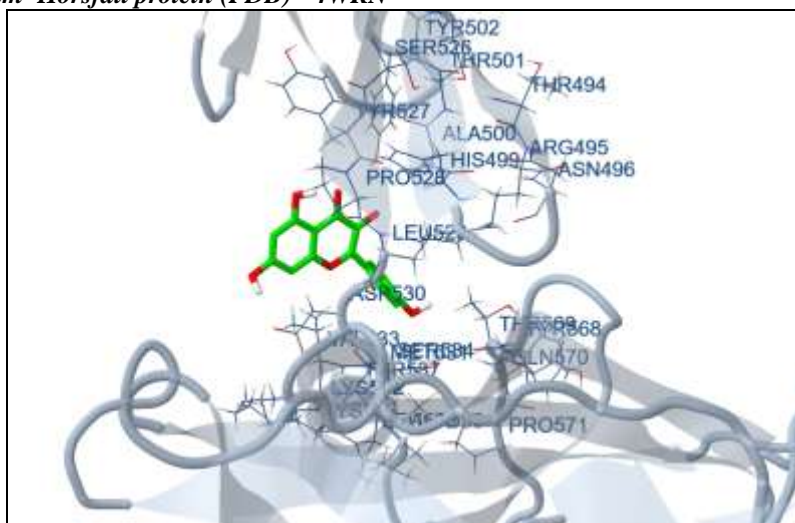
2D Interaction Plot Analysis



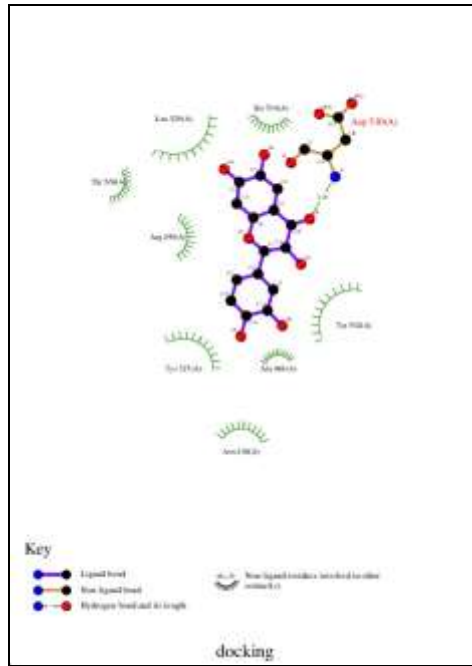
Hydrogen bond plotting with core amino acid Analysis



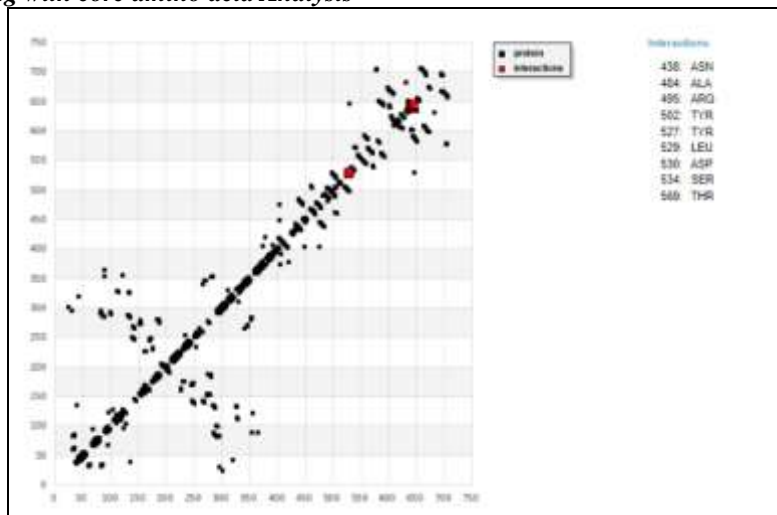
Kaempferol with Tamm-Horsfall protein (PDB) - 4WRN



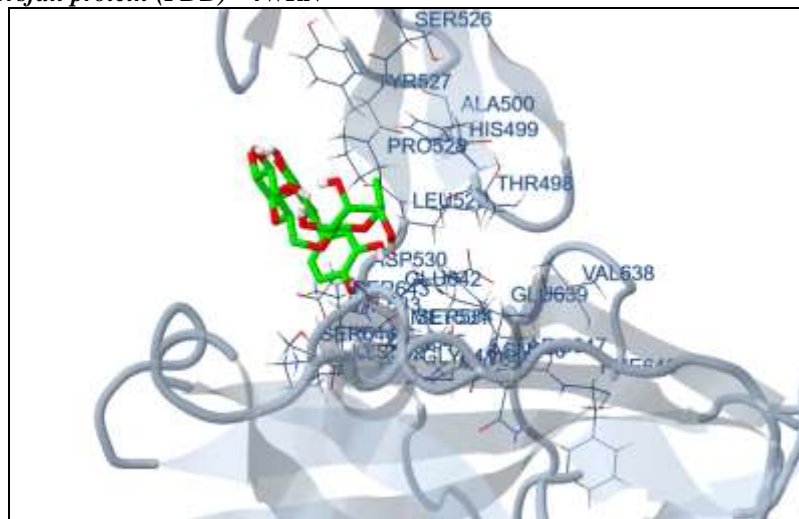
2D Interaction Plot Analysis



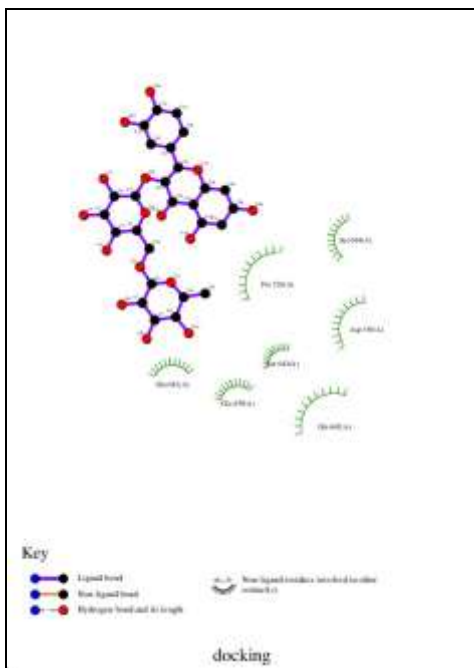
Hydrogen bond plotting with core amino acid Analysis



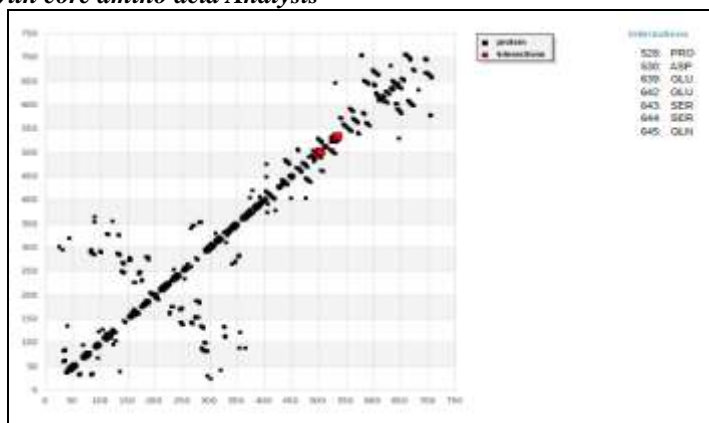
Rutin with Tamm–Horsfall protein (PDB) - 4WRN



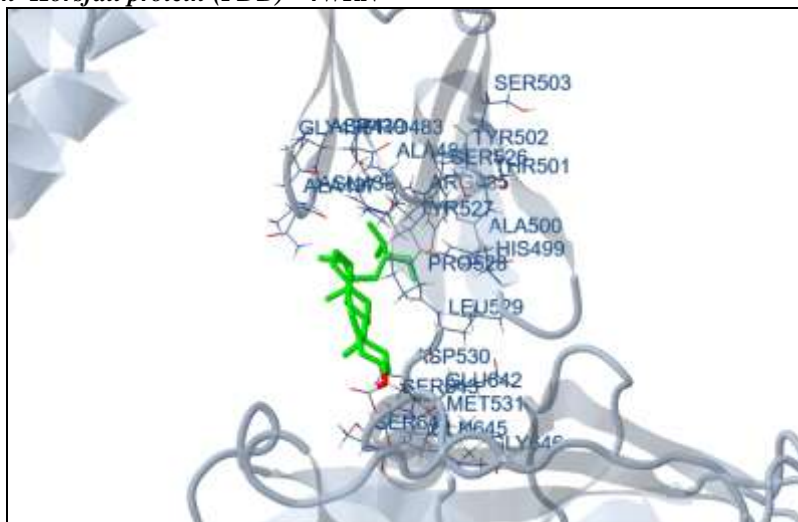
2D Interaction Plot Analysis



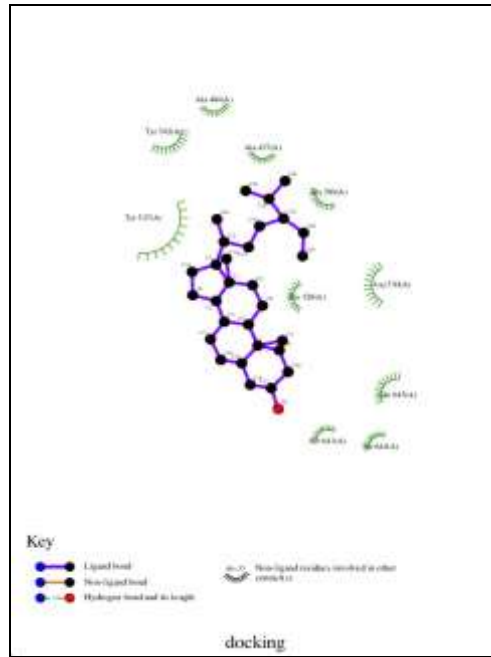
Hydrogen bond plotting with core amino acid Analysis



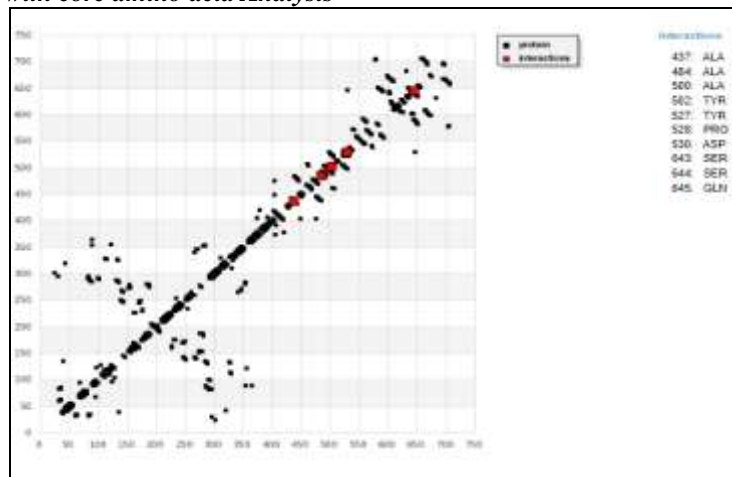
β -sitosterol with Tamm–Horsfall protein (PDB) - 4WRN



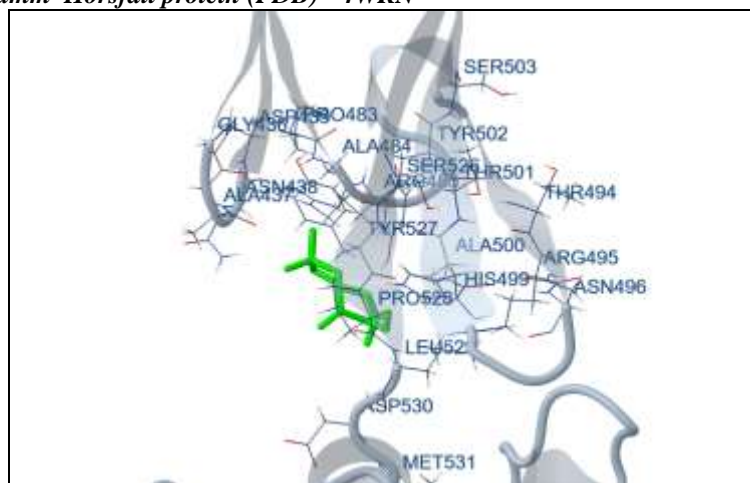
2D Interaction Plot Analysis



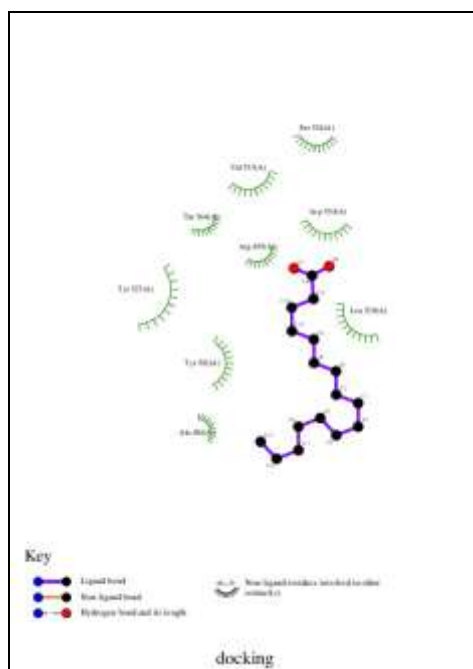
Hydrogen bond plotting with core amino acid Analysis



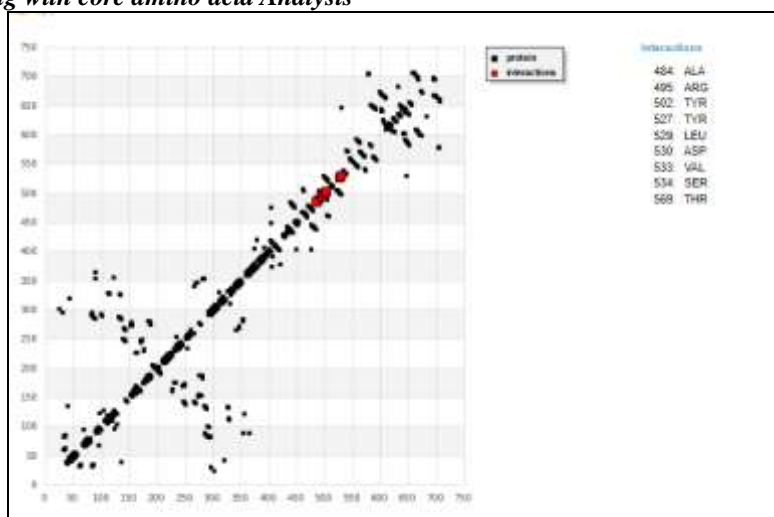
β -caryophyllene with Tamm–Horsfall protein (PDB) - 4WRN



2D Interaction Plot Analysis



Hydrogen bond plotting with core amino acid Analysis



RESULT AND DISCUSSION

Total of 7 bioactive lead compounds were retrieved from the herbs present in the siddha formulation. From reported data of the herb, the phytochemicals such as Kaempferol, Quercetin, β -sitosterol, β -caryophyllene and Palmitic acid present in the siddha formulation Kalladaipu Kudineer possess maximum of 2-4 interactions with the core active amino acid residues present on the target protein Tamm–Horsfall protein.

CONCLUSION

Based on the results of the computational analysis it was concluded that the bio-active compound's like Kaempferol, Quercetin, β -sitosterol, β -caryophyllene and Palmitic acid present in the herbal ingredients of siddha formulation Kalladaipu Kudineer 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 promising anti-urolithiasis activity by preventing calcium oxalate crystallization. Thereby phytochemicals 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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