International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 10, 2020
ISSN: 1475-7192

Revealing the Anti- Urolithiatic activity of Siddha poly herbal formulation *Samsakra Choornam* (SC) using In-silico Docking Technique

[1]*Thiruvancheeswaran Soruban, [2]Thanigainathan A, [3]Aravind Samy M

Abstract

Background: *Urolithiasis is the major cause of morbidity, and its prevalence is increasing in the world. Siddha formulas have managed pathogenic infections for ages. Siddha practice strengthens the host's immunity and resilience to pathogens.*

Aim*: The main aim of the present investigation is to screen the anti- urolithiatic activity of the Siddha poly herbal formulation Samsakra Chooranam through the In-silico docking technique.*

Materials and Methods: *Binding of phytocomponents 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 the management of urolithiasis and related symptoms.*

Results*: A total of 12 bioactive lead compounds were retrieved from the herbs present in the siddha formulation Samsakra Chooranam. From the reported data of the herb, the phytochemicals such as Glycyrrhetic acid, Liquiritin and Limonene possess maximum of three interactions with the core active amino acid residues present on the target protein Tamm–Horsfall protein.*

Conclusion: *From the results of the present in-silico screening, we have concluded that the phytochemicals of the siddha formulation SC display strong anti- urolithiatic activity by blocking the target enzyme and this trial drug can be recommended further for the clinical management to renal stone.*

Keywords: *Siddha, Docking, In-silico, Anti- urolithiatic, Samsakra Choornam, Renal stone*

INTRODUCTION

Kidney stone disease is a crystal concretion formed usually within the kidneys (1). It is one of the major cause of morbidity, its prevalence also increasing in the world and affects approximately 1–15% of the world's population. (2) About 1% - 19.1% of the population suffer from Kidney stone in Asia. (3) Classification of Kidney stones are calcium oxalate, calcium phosphate, uric acid, cysteine, struvite, and mixed stones types, there are depending on the material of the stones. Calcium stones account for almost 70–80% of all kidney stones. (4)

In Siddha systems, the disease is classified as 4,448 types. Among these *Kalladaippu* is one of the disease and this term also denoting Kidney stone. (5) The classical Siddha text book *Yugi vaidhya chinthamani 800* elaborately discussed about the etiology, pathology, classification, clinical features, and prognosis of *Kalladaippu*.(6) *Samsakra Chooranam* (SC) a poly herbal based preparation mentioned in classical Siddha literature *Pathartha Guna Vilakkam* (*Moola varkkam*), *Kannuswamy Pillai. C*, indicated for *Kalladaippu* (7).

However, there were no sufficient scientific evidence to prove the drug for Kidney Stone management. So, this study had been selected to evaluate the Anti- urolithiatic property through the In-silico docking technique.

OBJECTIVE:

Screening the Anti- urolithiatic activity of *Samsakra Choornam* (SC) using In-silico Docking Technique

[1]* Medical Officer, Siddha Teaching Hospital, Kaithady, Jaffna, Sri Lanka Email id: sorruthiru@gmail.com.
[2,3]PG Scholar, Dept. of Maruthuvam, National Institute of Siddha, Chennai 600047

MATERIALS AND METHODS:

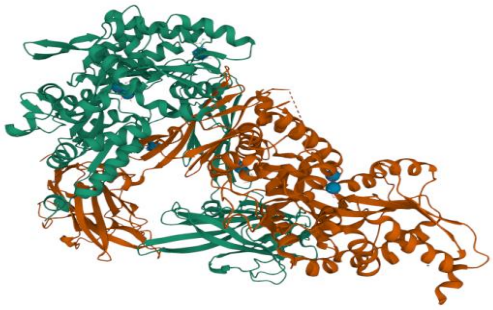
Methodology

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(8,9,10). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program(8,9,10). 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 (11). 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.

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.

Figure No 1: 3D- Structure of Tamm–Horsfall protein (PDB) - 4WRN



RESULTS

Ingredients of the *Samsakra Chooranam* (SC)

Malli (*Coriandrum sativum*) - 6 *Palam* (210g)
Adhimadhuram (*Glycyrrhiza glabra*- 1 *Palam* (35g)
Seeragam (*Cuminum cyminum*) - 1 *Palam* (35g)
Karunseeragam (*Nigella sativa*) - 1 *Palam* (35g)
Sathakuppai (*Anethum sowa*) - 1 *Palam* (35g)
Kirambu (*Syzygium aromaticum*) - 1 *Palam* (35g)
Sanna IlavangaPattai (Cinnamomum verum) - 1 *Palam* (35g)
Sarkarai (*Sacchrum officinarum*) - Sufficient Quantity

Dosage and Adjuvants: 1 to 2 gms. with honey or milk after food twice a day

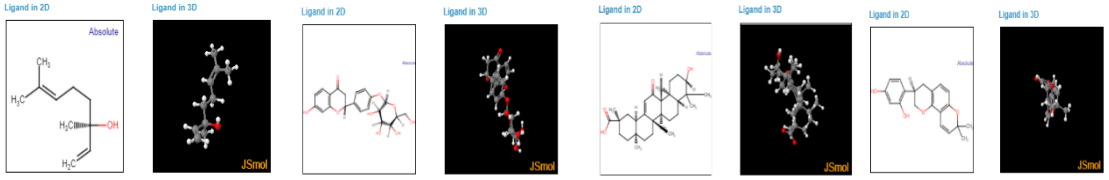
Indications : *Udal soodu* (Pitha disorder), *Nalirsuram* (Fever with shivering), *Ajiranam* (Indigestion), *Paithiyam*, *Sithabrammai* (Mental disorders), *Vanthi* (Vomitting), *Vikkal* (Hiccough), *Navaratchi* (Dryness of mouth), *Thathunattam* (Spermatorrhoea), *Peru eppam* (Belching), *Vai konuthal* (Facial paralysis) *Iduppu vali* (Hip pain), *Pakkavatham* (Hemiplegia) *Sirasu Noikal* (Cephalic disorders), *Thalaivali* (Headache), *Kirukiruppu* (Giddiness), *Nenju Erichal* (Acid peptic disorder), *Kannil Neervadithal* (Shedding of tears from the eye), *Parvai Mantham* (Sight disorders of the eye), *Ulkaichal* (Chronic Fever), *Kalladaippu* (Renal Stones)

Table No 1: List of Phytocomponents Selected for docking

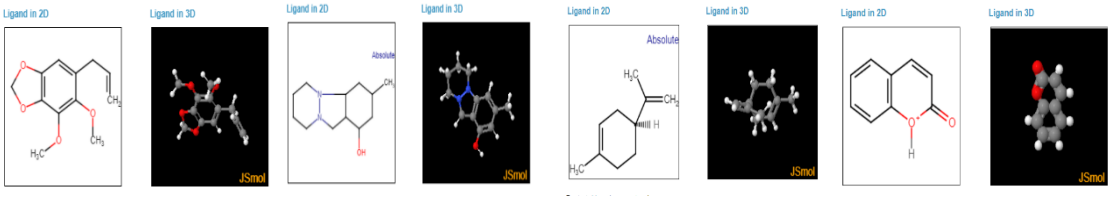
Medicinal plants	Pharmacological properties	References
Glycyrrhiza glabra	Glabrin Glycyrrhizic acid Liquiritin	Pastorino G, Cornara L, Soares S, Rodrigues F, Oliveira MBPP. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytother Res. 2018;32(12):2323-2339.
Cuminum cyminum	Linalool Coumaric acid Limonene	Ali Esmail Al-Snafi. The pharmacological activities of Cuminum cyminum - A review. IOSR Journal of Pharmacy. 2016;6(6): 46-65
Nigella Sativa	Nigeglaine	Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F. Nigella sativa L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. Evid Based Complement Alternat Med. 2019;2019:1528635. Published 2019 May 12. doi:10.1155/2019/1528635
Anethum Sowa	Apiole α-thujone	M. Moshfekus Saleh. Chemical Constitutents of essential oil from Anethum Sowa growing in Bangladesh. Bangladesh J. Sci. Ind. Res. 45(2), 173-176, 2010
Syzygium Aromaticum	β-caryophyllene Eugenol	Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules. 2020;10(2):202.
Coriandrum Sativum	Thiamine	Alev Önder. Coriander and Its Phytoconstituents for the Beneficial Effects.Potential of essential oils. DOI: 10.5772/intechopen.78656
Cinnamomum verum	β-caryophyllene Eugenol	Liyanage T, Madhujith T, Wijesinghe KG. Comparative study on major chemical constituents in volatile oil of true cinnamon (Cinnamomum verum Presl. syn. C. zeylanicum Blum.) and five wild cinnamon species grown in Sri Lanka.

Figure No 2: 2D and 3D Structure of Phytocomponents

2. 1. Linalool **2. 2. Liquiritin** **2. 3. Glycyrrhetic acid** **2. 4. Glabridin**



2. 5. Apiole **2. 6. Nigeglanine** **2. 7. Limonene** **2. 8. Coumaric acid**



2. 9. β Caryophyllene **2. 10. Thiamine** **2. 11.Eugenol** **2. 12. α-thujone**

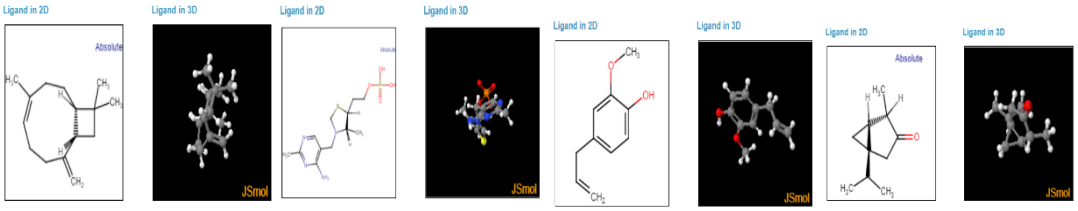


Table No 2: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Glabridin	324.40 g/mol	$C_{20}H_{20}O_4$	2	4	1
Glycyrrhetic acid	470.70 g/mol	$C_{30}H_{46}O_4$	2	4	1
Liquiritin	418.40 g/mol	$C_{21}H_{22}O_9$	5	9	4
Linalool	154.25 g/mol	$C_{10}H_{18}O$	1	1	4
Coumaric Acid	164.16 g/mol	$C_9H_8O_3$	2	3	2
Limonene	136.23 g/mol	$C_{10}H_{16}$	0	0	1
Nigeglanine	202.25 g/mol	$C_{12}H_{14}N_2O$	0	3	0
Apiole	222.24 g/mol	$C_{12}H_{14}O_4$	0	4	4
Alpha-Thujone	152.23g/mol	$C_{10}H_{16}O$	0	1	1
Eugenol	164.20 g/mol	$C_{10}H_{12}O_2$	1	2	3
Thiamine	265.36g/mol	$C_{12}H_{17}N_4O+$	2	5	4
β Caryophyllene	204.35 g/mol	$C_{15}H_{24}$	0	0	0

Table No 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
Glabridin	-5.29 kcal/mol	132.65 uM	-0.34 kcal/mol	-6.23 kcal/mol	561.242
Glycyrrhetic acid	-6.17 kcal/mol	29.96 uM	-0.14 kcal/mol	-6.74 kcal/mol	578.445
Liquiritin	-6.71 kcal/mol	12.01 uM	-0.11 kcal/mol	-7.31 kcal/mol	739.294
Linalool	-4.26 kcal/mol	754.57 uM	-0.03 kcal/mol	-5.62 kcal/mol	428.034
Coumaric Acid	-4.41 kcal/mol	583.93 uM	-0.00 kcal/mol	-4.41 kcal/mol	376.164
Limonene	-4.60 kcal/mol	427.00 uM	-0.00 kcal/mol	-4.90 kcal/mol	404.603
Nigeglanine	-4.79 kcal/mol	310.01 uM	-0.05 kcal/mol	-5.08 kcal/mol	434.479
Apiole	-3.82 kcal/mol	1.58 mM	-0.01 kcal/mol	-4.99 kcal/mol	489.31
Alpha-Thujone	-4.67 kcal/mol	379.69 uM	-0.02 kcal/mol	-4.96 kcal/mol	401.226
Eugenol	-4.25 kcal/mol	761.97 uM	-0.02 kcal/mol	-4.63 kcal/mol	444.09
Thiamin	-9.02 kcal/mol	245.46 nM	-0.55 kcal/mol	-6.74 kcal/mol	562.039
β Caryophyllene	-6.26 kcal/mol	25.89 uM	-0.04 kcal/mol	-6.56 kcal/mol	490.91

Table No 4: Amino acid Residue Interaction of Lead and Standard against Tamm–Horsfall protein (PDB) - 4WRN

Compound	**Interactions**	**Amino acid Residues**									
Glabridin	2	484 ALA	500 ALA	502 TYR	527 TYR	528 PRO	530 ASP				
Glycyrrhetic acid	3	500 ALA	502 TYR	527 TYR	528 PRO	529 LEU	530 ASP				
Liquiritin	3	498 THR	499 HIS	500 ALA	502 TYR	527 TYR	528 PRO	529 LEU	530 ASP	531 MET	645 GLN	647 ARG
Linalool	2	529 LEU	534 SER	537 THR	538 ALA	570 GLN	571 PRO	573 GLN				
Coumaric acid	2	529 LEU	534 SER	537 THR	538 ALA	570 GLN	571 PRO	573 GLN				
Limonene	3	495 ARG	529 LEU	534 SER	537 THR	570 GLN	571 PRO					
Nigeglanine	2	495 ARG	496 ASN	497 GLU	529 LEU	534 SER	570 GLN					
Apiole	2	495 ARG	497 GLU	529 LEU	534 SER	537 THR	570 GLN	571 PRO	573 GLN			
α-thujone	2	495 ARG	529 LEU	534 SER	537 THR	569 THR	570 GLN					
Eugenol	2	495 ARG	529 LEU	534 SER	537 THR	538 ALA	564 GLN	570 GLN	571 PRO	573 GLN		
Thiamine	2	495 ARG	529 LEU	534 SER	537 THR	538 ALA	539 LEU	570 GLN	571 PRO	573 GLN		
β Caryophyllene	2	495 ARG	500 ALA	502 TYR	527 TYR	529 LEU	530 ASP	533 VAL				

Figure No 3.1: Docking Pose Glabridin with Tamm–Horsfall protein (PDB) - 4WRN and2D Interaction Plot Analysi

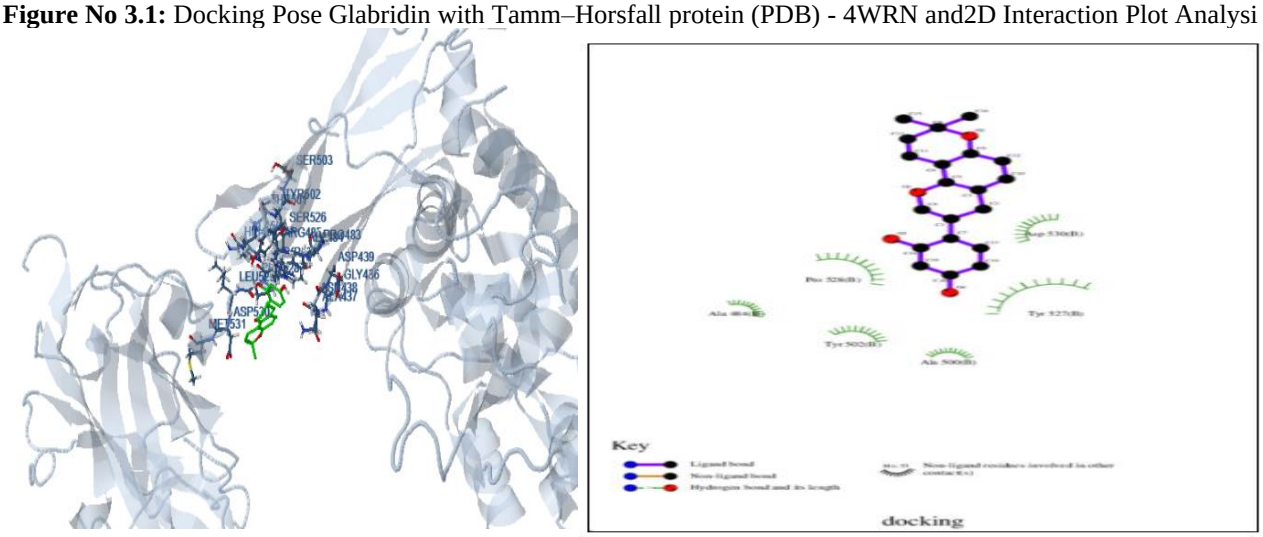


Figure No 3.2: Docking Pose Glycyrrhetic acid with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

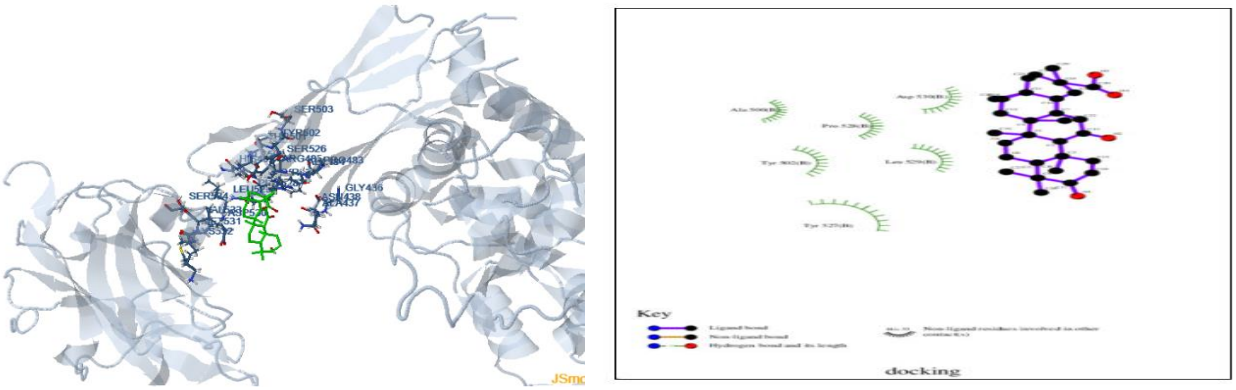


Figure No 3.3 : Docking Pose Liquiritin with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

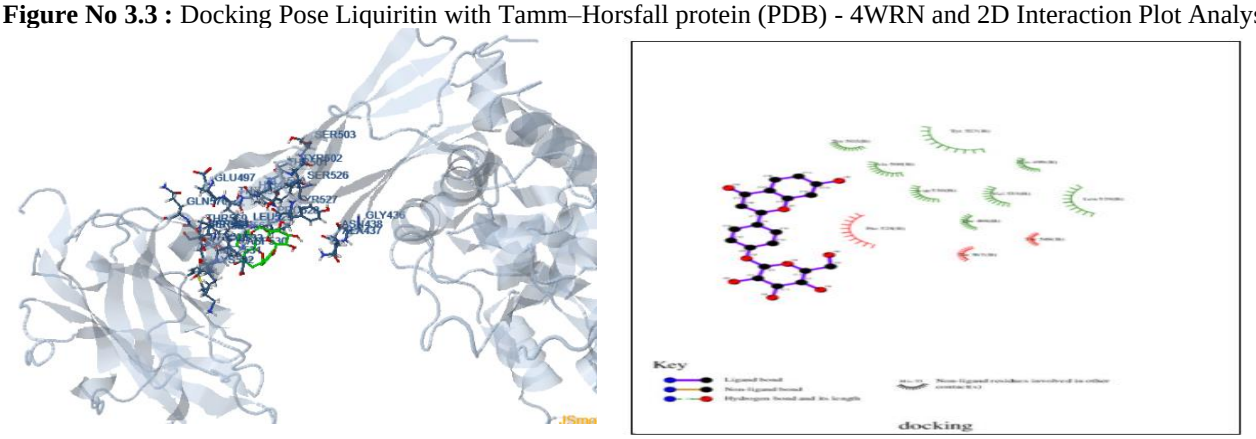


Figure No 3.4 : Docking Pose Linalool with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

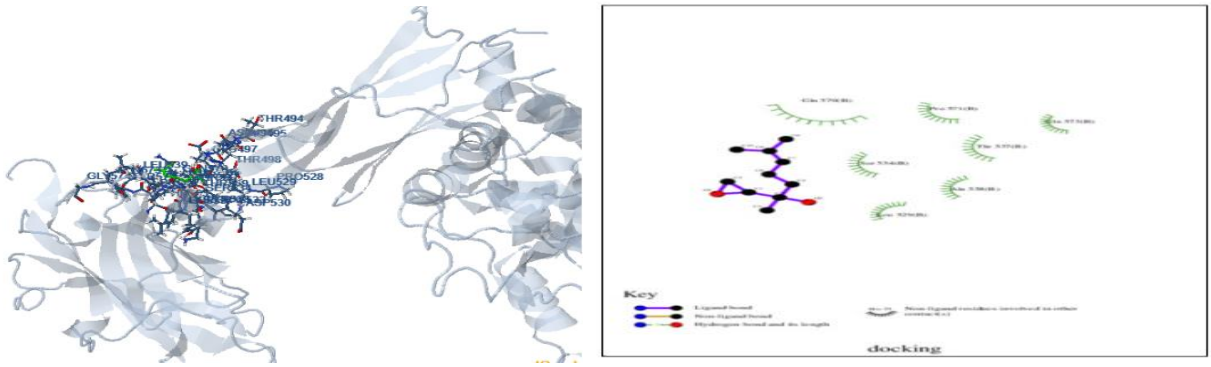


Figure No 3.5 : Docking Pose Coumaric Acid with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

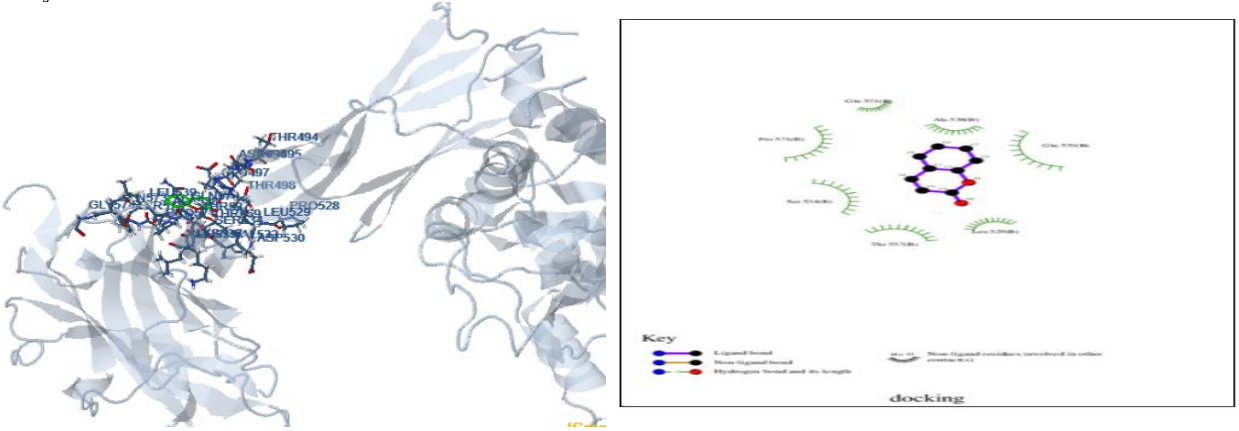


Figure No 3.6 : Docking Pose Limonene with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

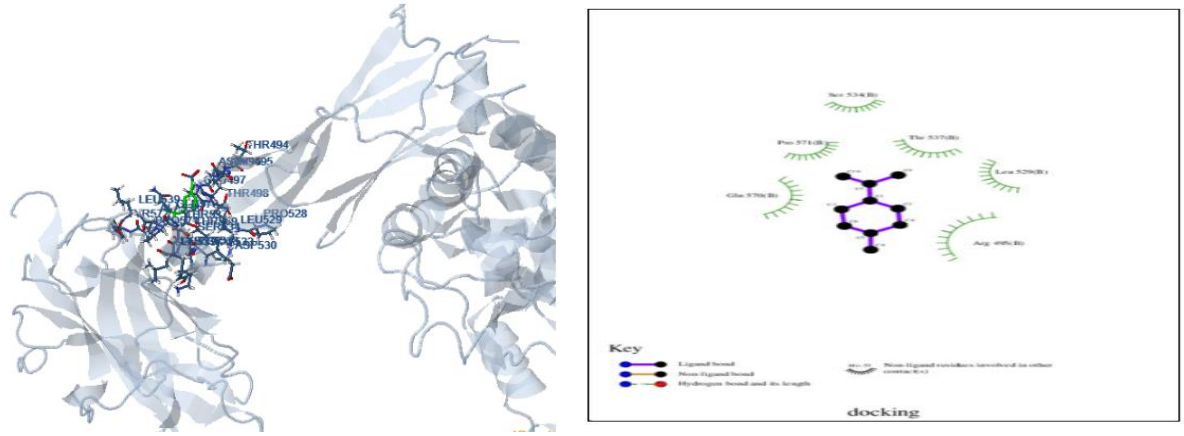


Figure No 3.7 : Docking Pose Nigeglanine with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

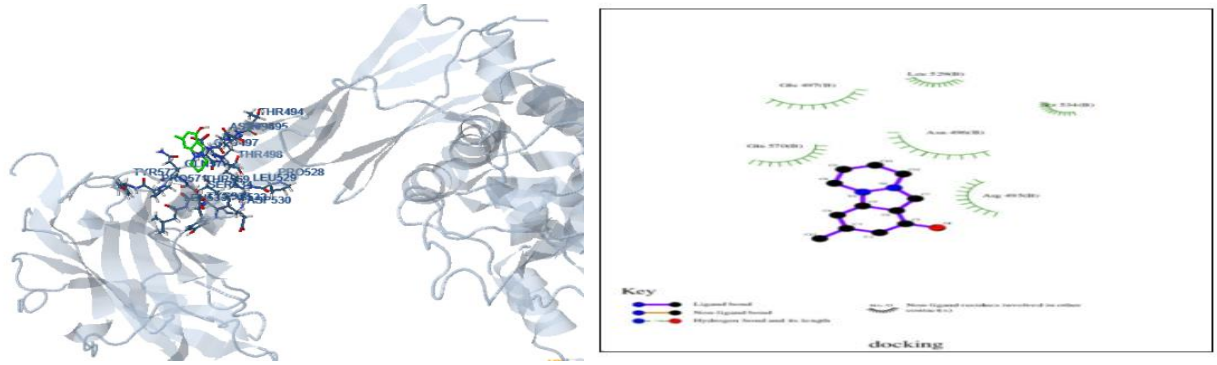


Figure No 3.8 : Docking Pose Apiole with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

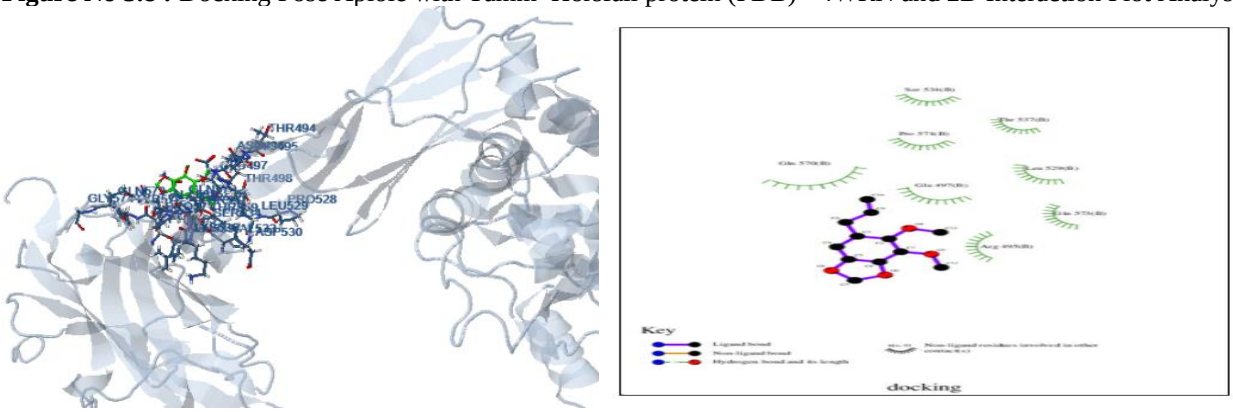


Figure No 3.9 : Docking Pose Alpha-Thujone with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

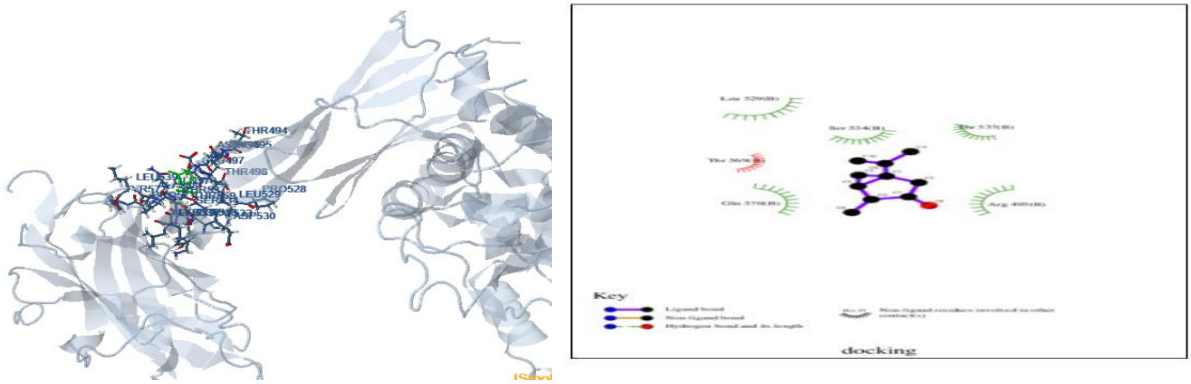


Figure No 3.10 : Docking Pose Eugenol with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

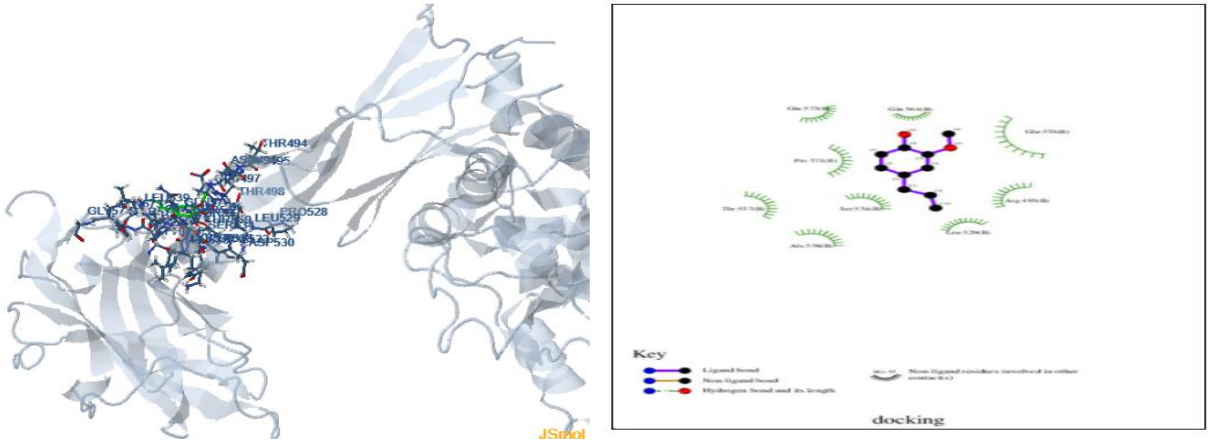


Figure No 3.11: Docking Pose Thiamine with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis

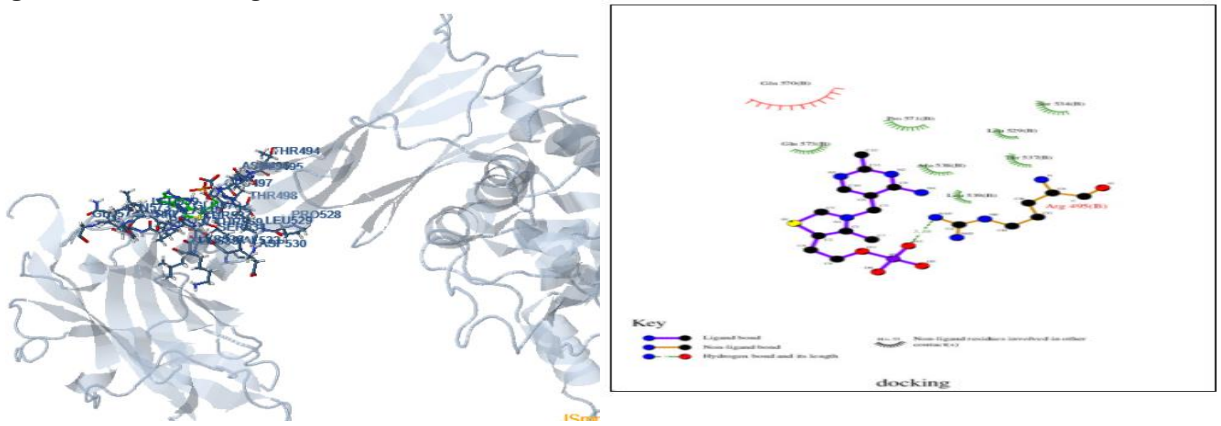
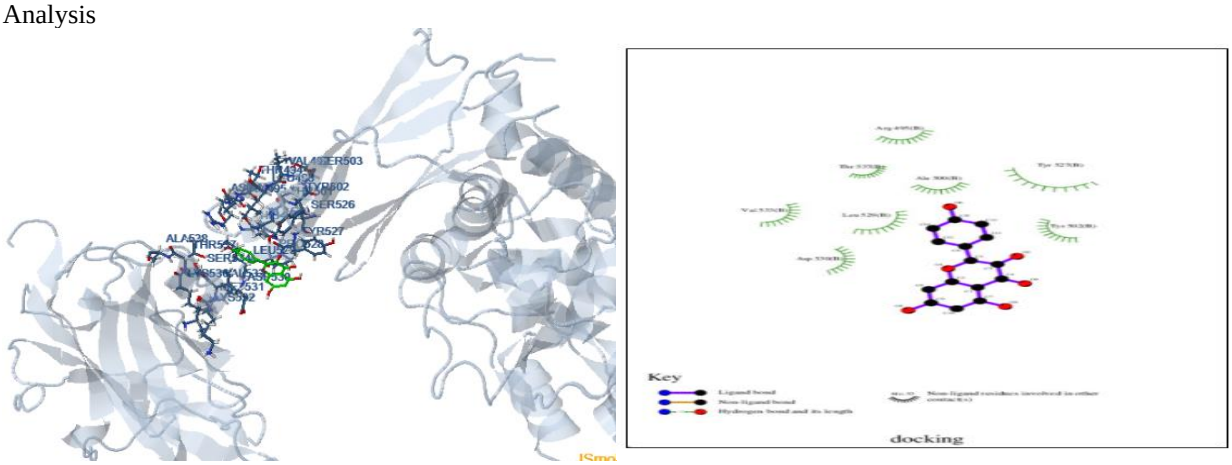


Figure No 3.12: Docking Pose β Caryophyllene with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis



DISCUSSION

Total of 12 bioactive lead compounds were retrieved from the herbs present in the siddha formulation *Samsakra Choornam*. They are Linalool, Liquiritin, Glycyrrhetic acid, Glabridin, Apiole, Nigeglanine, Limonene, Coumaric acid, β Caryophyllene, Thiamine, Eugenol and α-thujone. (Table No 1) From reported data of the herb, the phytochemicals such as Glycyrrhetic acid, Liquiritin and Limonene possess maximum of three interactions with the core active amino acid residues present on the target protein Tamm–Horsfall protein. Followed by this the compounds such as Glabridin, Linalool, Coumaric acid, Nigeglanine, Apiole, α-thujone, Eugenol, Thiamine and β Caryophyllene ranked second with the maximum of 2 interactions with the active site of the target Tamm–Horsfall protein.

Figure No: 2.1 Linalool, Figure No: 2.2. Liquiritin, Figure No: 2.3. Glycyrrhetic acid, Figure No: 2.4. Glabridin, Figure No: 2. 5. Apiole, Figure No: 2. 6. Nigeglanine, Figure No: 2. 7. Limonene, Figure No: 2. 8. Coumaric acid, Figure No: 2. 9. β Caryophyllene, Figure No: 2. 10. Thiamine, Figure No: 2. 11. Eugenol, Figure No: 2. 12. α-thujone represents

the 2D and 3D structure of Phtyocomponents of 12 compounds respectively. Table 1 shows Ligand properties of the compounds selected for docking analysis. Table 3 represents Summary of the molecular docking studies of compounds against Tamm–Horsfall protein (PDB) - 4WRN. Table 4 shows Amino Acid Residue Interaction of Lead against Tamm–Horsfall protein (PDB) - 4WRN. Figure No: from 3.1 to Figure No: 3.12 denotes docking pose of with Tamm–Horsfall protein (PDB) - 4WRN and 2D Interaction Plot Analysis respectively Glabridin, Glycyrrhetic acid, Liquiritin, Linalool, Coumaric Acid, Limonene, Nigeglanine, Apiole, Alpha-Thujone, Eugenol, Thiamine and β Caryophyllene.

Most of the ingredients of the trial drug also individually possess anti-urolithiatic activity. Rad AK et. al., carried out a study to determine whether the aqueous-ethanolic extract or the butanolic fraction of *Nigella sativa* (NS) seeds could prevent or reduce calculi aggregation in experimental calcium oxalate nephrolithiasis in Wistar rats. This study concluded N-butanol fraction and N-butanol phase remnant of NS showed a beneficial effect on calcium oxalate deposition in the rat kidney (19). E Sakhaee et al investigated the e protective effect of Cuminum cyminum (C. cyminum) essential oil on ethylene glycol induced nephrolithiasis in mice. It seems that C. cyminum essential oil significantly decreased formation of calcium oxalate crystals and the growth of renal calculi in different parts of the tubules (20).

Kayand N et. al., carried out a study to evaluate the diuretic activity of Glycyrrhiza glabra linn in experimental animals by following the standard procedure. Glycyrrhiza glabra linn increased the urine output in a dose dependent manner. However, it did not affect the urinary electrolyte concentrations. From the study, it can be concluded that the root of Glycyrrhiza glabra linn has diuretic property (21). Chandrasekaran S, et. al., investigated the anti-urolithiatic activity of aqueous and alcohol extracts of Coriandrum sativum L. by in vitro turbidity and titrimetric assays. The anti-urolithiatic activity was found to be more significant in the aqueous extract than in alcoholic extract of Coriandrum sativum L.(22) Sangi S.,et. Al., the experimentally evaluated the nephroprotective properties of Ginger (Zingiber officinale), Cinnamomum verum, and Nigella sativa in STZ induced diabetic rats. It is concluded that cinnamon has the potential to treat and protect the diabetic nephropathy.(23) Fujimoto et al. (2017) claimed that clove oil (*Syzygium Aromaticum*) induced blunts muscle contraction power and anaesthesia in three Amazon fish species: Pterophyllum scalare (angelfish), Heros severus (banded cichlid) and Parachheirodon axelrodi (cardinal tetra) (25)

CONCLUSION

Based on the results of the computational analysis it was concluded that the bio-active compound's like Glycyrrhetic acid, Liquiritin, Limonene, Glabridin, Linalool, Coumaric acid, Nigeglanine, Apiole, α-thujone, Eugenol, Thiamine and β Caryophyllene present in the siddha formulation *Samsakra Choornam* 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-urolithiatic 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 formulation *Samsakra Choornam* possess significant anti- urolithiasis activity.

REFERENCES

1. Alelign T, Petros B. Kidney stone disease: an update on current concepts. Advances in urology. 2018 Feb 4;2018.
2. Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. Rev Urol. 2010;12(2–3):e86.
3. Liu Y, Chen Y, Liao B, Luo D, Wang K, Li H, Zeng G. Epidemiology of urolithiasis in Asia. Asian journal of urology. 2018 Oct 1;5(4):205-14.
4. Nikpay S, Moradi K, Azami M, Babashahi M, Otaghi M, Borji M. Frequency of kidney stone different compositions in patients referred to a Lithotripsy Center in Ilam, West of Iran. J Pediatr Nephrol. 2016;4(3):102–7
5. Shanmugavelu M, H.B.I.M, Noi Nadal Noi Mudhal Nadal Thirattu, Part – I, Indian Medicine and Homeopathy, Fourth Edition 2006, Pg. 72-75
6. Anonymous, Yugimunivar Vaithya Chinthamani, Peru noi 800, Part 1, published by Committee of Siddha Book Publication, 2nd Edition, 1976. P 426 - 431
7. Kannuswamy Pillai. C, Pathartha Guna Vilakkam (Moola varkkam), B Rathinayagar and Son, 1936, P 286
8. Bikadi, Z., Hazai, E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J. Cheminf. 1, 15 (2009)
9. T. A. Halgren. *Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94*. Journal of Computational Chemistry 17 (5-6), 490-519 (1998)
10. G. M. Morris, D. S. Goodsell, et al. *Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function*. Journal of Computational Chemistry 19 (14), 1639-1662(1998)
11. F. J. Solis and R. J. B. Wets. *Minimization by Random Search Techniques*
12. Pastorino G, Cornara L, Soares S, Rodrigues F, Oliveira MBPP. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytother Res. 2018;32(12):2323-2339
13. Ali Esmail Al-Snafi. The pharmacological activities of Cuminum cyminum - A review. IOSR Journal of Pharmacy. 2016;6(6): 46-65

14. Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F. Nigella sativa L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. Evid Based Complement Alternat Med. 2019;2019:1528635. Published 2019 May 12. doi:10.1155/2019/1528635
15. M. Moshfekus Saleh. Chemical Constitutents of essential oil from Anethum Sowa growing in Bangladesh. Bangladesh J. Sci. Ind. Res. 45(2), 173-176, 2010
16. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules. 2020;10(2):202.
17. Alev Önder. Coriander and Its Phytoconstituents for the Beneficial Effects.Potential of essential oils. DOI: 10.5772/intechopen.78656
18. Liyanage T, Madhujith T, Wijesinghe KG. Comparative study on major chemical constituents in volatile oil of true cinnamon (Cinnamomum verum Presl. syn. C. zeylanicum Blum.) and five wild cinnamon species grown in Sri Lanka.
19. Rad AK, Rajaei Z, Tehranipour M, Monavar N. The preventive effect of N-butanol fraction of Nigella sativa on ethylene glycol-induced kidney calculi in rats. Pharmacognosy magazine. 2011; 1;7(28):338
20. Sakhaee E, Kheirandish R, Eshaghi S. Protective effects of Cuminum cyminum L. essential oil on ethylene glycol induced nephrolithiasis in mice. Journal of Coastal Life Medicine. 2016;4(5):403-5..
21. Kayande N, Kushwah P. Evaluation of Diuretic Activity of Glycyrrhiza Glabra Linn in Experimental Animals. PharmaTutor. 2014 Jun 1;2(6):167-70.
22. Chandrasekaran S, Veerasamy V. Anti-urolithiatic activity of whole plant aqueous and ethanolic extract of Coriandrum sativum L.-an in vitro approach. Malaya Journal of Biosciences (MJB). 2018 Apr 17;5(1):28-36.
23. Sangi S, Elwahab M. Experimental evaluations of the nephroprotective properties of ginger (Zingiber officinale), Cinnamomum verum and Nigella sativa in STZ induced diabetic rats. International Journal of Biology, Pharmacy and Allied Sciences. 2017;6(6):1195-209.
24. Fujimoto RY, Pereira DM, Silva JCS, de Oliveria LCA, Inoue LAKA, Hamoy M et al. Clove oil induces anaesthesis and blunts muscle contraction power in three Amazon fish species. Fish Physiology and Biochemistry. 2017; 44:245-256