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IN SILICO MOLECULAR DOCKING ANALYSIS OF PHYTOCONSTITUENTS OF ANGAYA CHOORANAM AGAINST ULCER TARGET H PYLORI UREASE

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Abstract

Background: Angaya Chooranam is a Siddha classical formulation that has been analyzed for its potential role in the management of Peptic Ulcer Disease (PUD). Peptic Ulcer Disease is strongly linked to chronic colonization by Helicobacter pylori (H. pylori). The bacterial enzyme urease plays a pivotal role in survival and pathogenesis by neutralizing gastric acid through urea hydrolysis. Rising antibiotic resistance against conventional therapies necessitates alternative therapeutic candidates. Angaya Chooranam is a traditional Indian Siddha polyherbal formulation widely prescribed for gastrointestinal disorders, yet its molecular level mechanisms against H. pylori targets remain unexplored.
Objective: This study evaluated the in silico inhibitory potential, binding affinities, and molecular interactions of key phytoconstituents present in the Angaya Chooranam against the catalytic domain of H. pylori urease (PDB ID: 1E9Y).
Methods: Bioactive phytoconstituents were retrieved from the Angaya Chooranam. The 3D crystal structure of H. pylori urease was retrieved from the Protein Data Bank, prepared by removing water molecules and heteroatoms, and adding missing hydrogen atoms. Molecular docking studies were performed using AutoDock Vina. The core amino acids targeted were
LYS-445 and VAL-473, TYR-475.
Results: The computational analysis demonstrated that selected phytochemicals, including Ferulic acid, Violaxanthin, Thymol, Quercetin, Ajoene, exhibited 2 to 3 significant interactions with the core active amino acid residues of H pylori Urease. Theses interactions suggest a potential therapeutic agent for the management of peptic ulcer disease.
Conclusion: The findings provide computational validation for the traditional clinical use of Angaya Chooranam in peptic ulcer management. The identified lead phytochemicals represent promising natural urease inhibitors that permit further in vitro and in vivo experimental validation for novel anti-ulcer drug development.

Keywords: Siddha Medicine, Angaya Chooranam, Helicobacter pylori, Urease Inhibitor, Molecular Docking, Peptic Ulcer Disease.

INTRODUCTION:

Siddha medicine is one of the oldest traditional systems of medicine practiced in India, predominantly in the southern state of Tamil Nadu. It is believed to have been founded by the Siddhars, enlightened sages who developed this holistic medical system based on the principles of maintaining health, preventing disease, and attaining longevity through the preservation and perfection of the human body.

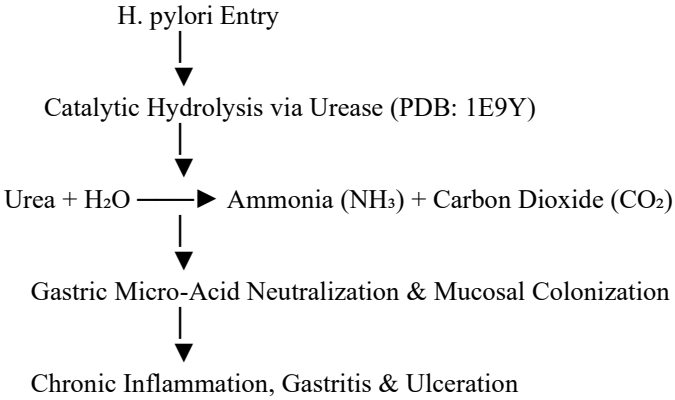
The World Health Organization has considered H. pylori as a class 1 carcinogen.[1] A recent study reported that until 2015, around 4.4 billion people were infected with H. pylori, and the prevalence rate in Africa, Latin America and Asia is substantially increasing.[2] Thus, H. pylori is a global public health concern.

Peptic Ulcer Disease (PUD) remains a major global healthcare concern, characterized by mucosal erosions in the stomach and duodenum driven by an imbalance between mucosal protective factors and aggressive luminal agents. Among the primary risk factors, chronic infection by the Gram-negative microaerophilic bacterium Helicobacter pylori is established as the principal cause of gastritis, peptic ulcers, and gastric

adenocarcinomas. A critical virulence factor enabling H. pylori to survive in the hostile, highly acidic gastric environment is its nickel-dependent enzyme, urease.

Urease catalyzes the rapid hydrolysis of urea into ammonia and carbon dioxide. The generated ammonia neutralizes ambient gastric acid, creating a protective micro-environment around the bacterium that allows successful colonization, migration across the gastric mucus layer, and subsequent inflammation of the host tissue.

The high-resolution crystal structure of H. pylori urease (PDB ID: 1E9Y) reveals a complex molecular architecture featuring an active binding pocket containing two catalytic nickel ions. Inhibition of this enzyme directly impairs H. pylori survival and colonization, making the urease catalytic domain a prime target for anti-ulcer drug discovery.



Although standard triple and quadruple therapy regimens combining proton pump inhibitors (PPIs) with antibiotics such as amoxicillin and clarithromycin are widely utilized, their clinical efficacy is increasingly challenged by rising antibiotic resistance, adverse drug reactions, high relapse rates, and poor patient compliance. Consequently, there is an urgent demand for novel, effective, and safe anti-ulcer agents derived from traditional medicine systems.

The Siddha medicine offers a rich repertoire of polyherbal and herbomineral formulations targeting gastrointestinal ailments. Angaya Chooranam is a classical Siddha polyherbal formulation traditionally prepared as per the Siddha literature Marunthu Sei Iyalum Kalaiyum indicated for digestive disorders, gastritis, hyperacidity, peptic ulcers, and post-partum recovery. ANGAYAM is a thogai sarakku and it comprises several bioactive botanical ingredients, including Mustard (Kadugu), Asafoetida (Perungayam), Fenugreek (Vendhayam), Garlic (Vellai poondu) and Ajwain (Omam). In traditional preparation method dried Neem flowers (Vepam poo), dried Turkey berries (Sundai vatral), and dried black night shade fruits (Manathakkali) are added to it. [3]

Despite its extensive historical and clinical usage, the precise molecular mechanisms by which Angaya Chooranam exerts its gastroprotective and anti-ulcer effects remain to be comprehensively mapped at the structural biology level. Computational molecular docking provides an efficient in silico approach to screen and evaluate the binding affinities, interaction profiles, and active-site dynamics of candidate phytochemicals against key therapeutic target enzymes.

Therefore, this study investigates the potential in silico anti-ulcer mechanism of major active constituent molecules identified in Angaya Chooranam against the H. pylori urease enzyme (PDB: 1E9Y), aiming to provide computational evidence validating its traditional clinical claims and identifying novel botanical lead compounds for anti-urease therapy.

Aim and Objective:

The present study is aimed to accomplish in Silico computational binding of phytocomponents of Angaya Chooranam with the core amino acids (LYS-445 and VAL-473, TYR-475) of the targets by forming hydrogen bond will hinder the function of the target enzyme H pylori Urease with PDB – 1E9Y. The enzyme urease catalyses the hydrolysis of urea to ammonia. Urease has been reported to be a prominent virulence determinant of H pylori in the pathogenesis of ulcer. Thereby phytocomponents which inhibit the target viral enzyme H pylori urease may act as a potential therapeutic agent for management of ulcer.

MATERIALS AND METHODS:

Lead Molecules from Angaya Chooranam

Docking calculations were carried out for the compounds retrieved from the herbal sources such as Ferulic acid, Violaxanthin, Thymol, Quercetin, Ajoene possess with the core active amino acid residues present on the target enzyme enzyme H pylori Urease with PDB – 1E9Y. (Table.1 & Fig A). The ligand molecular properties are illustrated in Table. 2

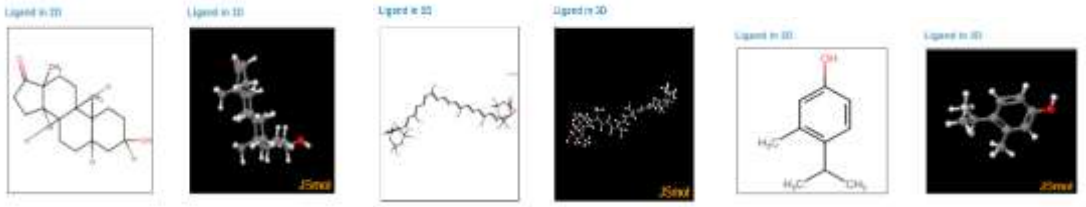
Table 1: List of Phytocomponents Selected for docking

Herb	Phyto components	Reference
Ferula asafetida	Ferulic acid	[4]
Brassica nigra	Violaxanthin	[5]
Trachyspermum ammi	Thymol	[6]
Trigonella foenum graecum	Quercetin	[7]
Allium sativum	Ajoene	[8]

The docking study report on Anti-Ulcer Activity

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

Ferulic acid **Violaxanthin** **Thymol**



Quercetin **Ajoene**

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
Ferulic acid	194.186 g/mol	$C_{10}H_{10}O_4$	2	4	3
Violaxanthin	600.9 g/mol	$C_{40}H_{56}O_4$	2	4	10
Thymol	150.221 g/mol	$C_{10}H_{14}O$	1	1	1
Quercetin	302.23 g/mol	$C_{15}H_{10}O_7$	5	7	1
Ajoene	234.4 g/mol	$C_9H_{14}OS_3$	0	4	8

Target details and receptor structure:

Crystalline structure of the target enzyme H pylori Urease (PDB) - 1E9Y 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
1E9Y	H pylori Urease

3D- Structure of H pylori Urease (PDB) - 1E9Y

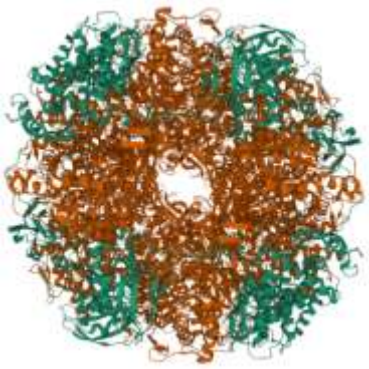


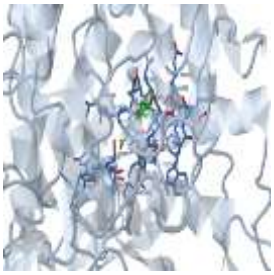
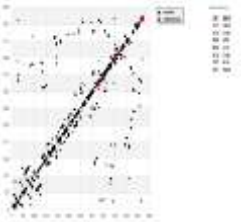
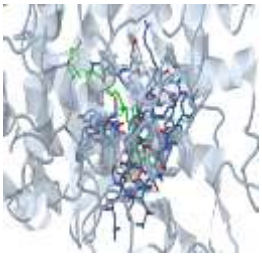
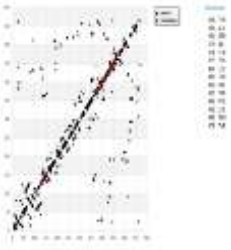
Fig B: Receptor Structure

Tool for study

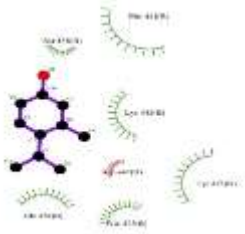
Methodology: [9, 10, 11, 12]

Docking calculations were carried out for retrieved phytocomponents against target enzyme CYP- 17α-hydroxylase. 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.

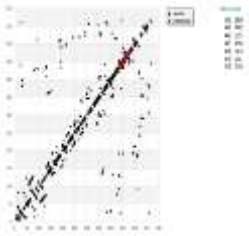
Figure No: C Docking pose

Ferulic acid with H pylori Urease with PDB 1E9Y	**2D Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**
		
Violaxanthin with H pylori Urease with PDB 1E9Y	**2D Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**
		
Thymol with H pylori Urease with PDB 1E9Y	**2D Interaction Plot Analysis**	**Hydrogen bond plotting with core amino acid Analysis**

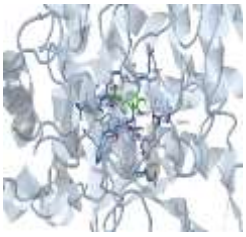
Quercetin with H pylori Urease with PDB 1E9Y



2D Interaction Plot Analysis

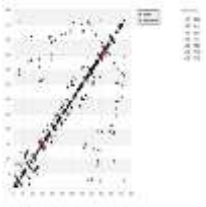


Hydrogen bond plotting with core amino acid Analysis

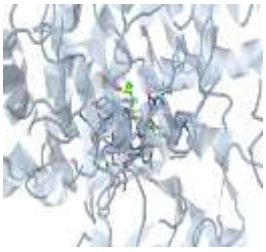


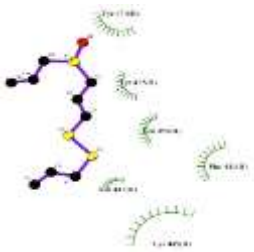
Ajoene with H pylori Urease with PDB 1E9Y

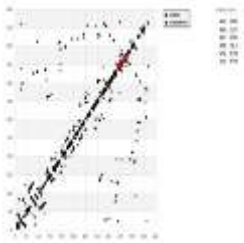
2D Interaction Plot Analysis



Hydrogen bond plotting with core amino acid Analysis







RESULTS AND DISCUSSION:

Total of 5 bioactive lead compounds were retrieved from the herbs present in the siddha formulation Angaya Chooranam. From reported data the phytochemicals such as Ferulic acid, Ajoene, Thymol and Violaxanthin reveals maximum of 2-3 interactions that accounts of 75-100 % of the occupancy with the core active amino acid residues present on the target protein enzyme H pylori urease. The key role of Helicobacter pylori urease is neutralizing stomach acid, enabling bacterial survival, and driving tissue damage. It converts urea into ammonia and carbon dioxide. Urease has been reported to be a prominent virulence determinant of H pylori in the pathogenesis of ulcer. Thereby phytocomponents which inhibit the target H pylori urease may act as a potential therapeutic agent for management of ulcer. H. pylori urease by interacting with active amino acid present on the active site. The interaction brings an idea that these phytochemicals may inhibit the above process. The molecular docking studies of the lead compounds against H pylori Urease with PDB 1E9Y, Amino acid Residue Interaction have been tabulated Table 3 & 4 and docking pose in Figure C.

Table 3: The molecular docking studies of compounds against H pylori Urease with PDB 1E9Y

Compounds	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Ferulic acid	-5.06 kcal/mol	193.89 uM	-0.57 kcal/mol	-5.60 kcal/mol	502.41
Violaxanthin	-5.54 kcal/mol	87.31 uM	-0.01 kcal/mol	-5.04 kcal/mol	507.579
Thymol	-5.11 kcal/mol	179.10 uM	-0.07 kcal/mol	-5.77 kcal/mol	447.151
Quercetin	-6.20 kcal/mol	28.67 Um	-0.26 kcal/mol	-5.49 kcal/mol	531.903
Ajoene	-4.15 kcal/mol	912.54 uM	-0.24 kcal/mol	-4.77 kcal/mol	410.552

Table 4: Amino acid Residue Interaction of Lead compounds against H pylori Urease with PDB 1E9Y

Compounds	Interactions	Amino acid Residues								
Ferulic acid	2	151 SER	371 GLU	374 THR	444 VAL	445 LYS	475 LYS	563 ALA	567 SER	
Ajoene	2	441 PHE	445 LYS	447 ASN	459 GLN	474 TYR	475 LYS			
Thymol	3	438 SER	441 PHE	445 LYS	447 ASN	459 GLN	473 VAL	475 LYS		
Quercetin	1	147 THR	150 ALA	151 SER	445 LYS	446 PRO	474 TYR	475 LYS		
Violaxanthin	2	129 THR	150 ALA	151 SER	373 ILE	374 THR	377 TRP	406 LEU	409 TYR	435 VAL
		437 TRP	442 PHE	445 LYS	446 PRO	475 LYS				

CONCLUSION:

For pharmacological validation of Angaya Choornam, the docking study is 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 Ferulic acid, Ajoene, Thymol and Violaxanthin present in the Angaya Chooranam reveals significant binding against the target enzyme H. pylori urease by interacting with active amino acid present on the active site thereby it was concluded that these compounds may exert pharmacological effects on anti-ulcer activity by inhibiting the enzyme H pylori of urease that catalyse the hydrolysis of urea to ammonia. Urease has been reported to be a prominent virulence determinant of H. pylori in the pathogenesis of ulcer. Thereby phytocomponents which inhibit the target H. pylori urease may act as a potential therapeutic agent for management of ulcer. It was concluded that the phytochemicals present in the present in the Siddha formulation Angaya Chooranam reveals significant anti-ulcer activity.

Acknowledgement

We would like to thank Noble Research Solutions, Perambur, Chennai for supporting this research work.

Authors Contribution

Conceptualization of the study, supervision and formal analysis were performed by Dr. S. Selvakumar, The original draft was prepared and validated by Dr. K. Nalina Saraswathi, while writing, editing, and methodology were conducted by Dr. M. Ramani. All authors have reviewed and approved the final version of the manuscript for publication.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Funding

Self-funded research work.

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