MOLECULAR DOCKING STUDY OF LEAD COMPOUNDS FROM ELATHY CHOORANAM A SIDDHA FORMULATION AGAINST HELICOBACTER PYLORI UREASE

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Abstract

Helicobacter pylori (H. pylori), infection has strong association with development of gastritis, peptic ulcers, and stomach cancer. Though there exist unclear and complex treatment modalities for H.Pylori infection, recent step-up in herbal medicines can be a way out for these intricate research arena. Several efforts have been taken off late to transform traditional medicine into a modern industry in order to deliver healthcare to the common people. The present study was aimed at identifying potential lead phytochemicals from traditional anti-ulcer Siddha medicine ElathyChooranam that can inhibit H. pylori and possibly serve as adjuvant treatments. Here, in silico molecular docking was performed using auto dock tools to identify potential inhibitors that favour the binding with the core amino acids (LYS-445 and VAL-473, TYR-475) which inturn would hinder the function of the enzyme H pylori Urease.Based on the results of the computational analysis it was concluded that the bio-active compound's like β-sitosterol , Kaempferol , Piperine, Abiesin, Gingerenone-A and Nerolidol present in the Siddha formulation ElathyChooranampossess significant binding against the target H pylori urease Thereby it can be concluded that these compounds may exert promising anti-ulcer activity by inhibiting the enzyme H pylori urease which is regarded as the predominant virulence determinant of H pylori that catalyze the hydrolysis of urea to ammonia in the pathogenesis of ulcer.

Keywords: ElathyChoranam, Kunmam, Traditional medicine, Herbal medicine, H. Pylori.

INTRODUCTION

H.Pylori, first identified in 1982 by Robin Warren and Barry Marshall.is one of the most common bacterial infection affecting more than 50% of the world's population [1]Though most infected people remain asymptomatic, 10 ~ 20% of H. pylori infection will eventually develop into chronic gastritis, peptic ulceration, mucosa-associated lymphoid tumors, or even gastric adenocarcinoma [2] The World Health Organisation has considered H. pylori as a class 1 carcinogen [3] Therefore, eradication of H. pylori is not only regarded as an effective treatment for gastritis, peptic ulcer disease but also has the potential to reduce the risk of gastric cancer development [4] Currently, the main treatment option for H. pylori is the standard triple therapy, combining two antibiotics clarithromycin and amoxicillin with one proton pump inhibitor omeprazole[5] But the use of higher doses or more drugs and the development of a drug-resistant strain has led to more than 20% rate of therapy failure and higher risks of side effects including DNA toxicity and complex interactions with liver metabolic enzymes. This has provoked some researchers to combine phytomedicines with triple therapy [6] In silico molecular docking is a an efficient method to screen bioactive compounds from a pool of phytochemicals using computational analysis. It aids to identify inhibitors from phytochemicals by analysing the interactions between the target protein and ligands.[7] Several molecular docking software programmes have been successfully applied in computational drug design (CADD). Docking can simulate the interactions between a ligand and protein, calculate their binding energies and predict the possibility of whether a compound may bind to a pharmacological target, such as an enzyme.[7]

Role of enzyme H. pylori Urease in the pathogenesis of gastrointestinal ulcers

H.Pylori urease is the most significant pharmacological targets among the three identified targets. H. pylori requires urease and the H+-gated urea channel to survive in the low pH environment of human gastric fluid. Ureases catalyses the hydrolysis of urea to ammonia., which neutralises the stomach acid and resists the damage caused by acidic environments [8]Binding of phytocomponents with the core amino acids (LYS-445 and VAL-473, TYR-475) of the target by forming hydrogen bond will hinder the function of the enzyme H pylori Urease with PDB – 1E9Y. The enzyme urease 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.

LITERATURE REVIEW ON ELATHYCHOORANAM

Table-1: Ingredients of Elathychooranam.[8]

S.No	Botanical Name	Tamil Name	Family
1	Eletteria cardamomum	Elam	Zingiberaceae
2	Cuminum cyminum	Seeragam	Apiaceae
3	Syzygiumaromaticum	Kirambu	Myrtaceae
4	Glycyrrhiza glabra	Adhimadhuram	Fabaceae
5	Emblica officinalis	Nellivattral	Euphorbiaceae
6	Cinnamomum tamala	Lavangapathiri	Lauraceae
7	Cinnamomum verum	Sirunagapoo	Lauraceae
8	Murrayakoenigii	Karuveppilai	Rutacea
9	Santalum album	Sandhanam	Santalaceae
10	Nardostachysjatamansi	Sadamanjil	Valerianaceae
11	Foeniculam vulgare	Sombu	Apiaceae
12	Saccharum officinarum	Karkandu	Poaceae

Elathychooranam a polyherbal Siddha formulation used as anti-ulcer siddha drug has been indicated for antiulcer activity in traditional Siddha text, Siddha vaithyathirattu. [9]

Literature review on each of the ingredients of ElathyChooranam has shown to have promising antiinflammatory activity and therefore can have gastroprotective effects by conserving the gastric barrier function[10] Therefore the study drug was aimed to be tested for its anti-H.Pylori action insilico. One of the downfalls of using plant extracts as medicine is the imprecise type and amount of the active ingredients. This is because many factors could affect the number of active ingredients of a plant, including climate, soil type and harvesting time [11] Hence, identifying the anti-H. pylori compounds in these plants may help to produce more predictable responses and accurate dosing regimens.

MATERIALS AND METHODS

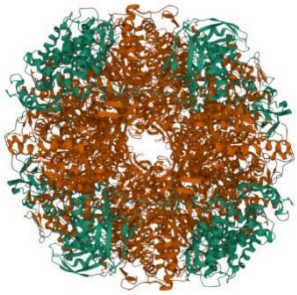
Methodology

Docking calculations were carried out for retrieved phytocomponents against target enzyme H pylori urease. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [12] Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program [13] 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 [13] 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.

List of Phytocomponents Selected for docking

Herb	Phyto components
Elettaria cardamomum	Nerolidol[14]
Zingiber offinarum	Gingerenone-A [15]
Abies Webbiana	Abiesin[16]
Mesua ferrea	β-sitosterol [17]
Piper nigrum	Piperine [18] [19]
Syzygiumaromaticum	Kaempferol [20]

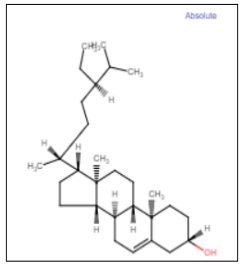
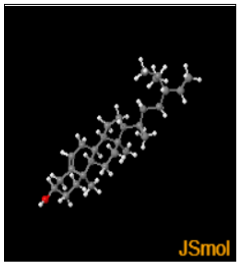
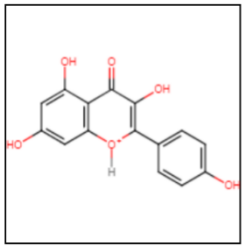
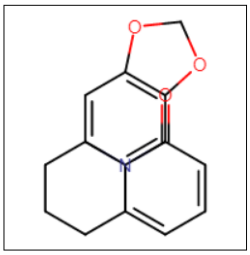
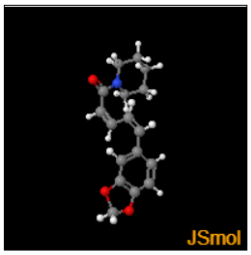
3D- Structure of H pylori Urease (PDB) - 1E9Y



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.[21]

Table-1. 2D and 3D structure of Phytocomponents of ElathyChooranam

S.no	Phytochemical	2D & 3D structure
1	Beta Sitosterol	 
2	Kaempferol	 
3	Piperine	 

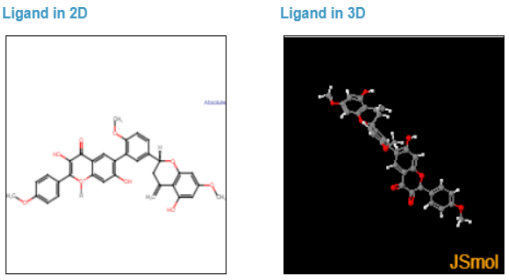
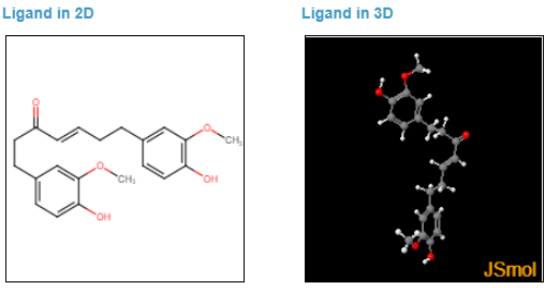
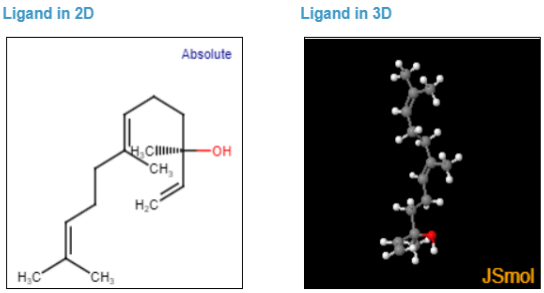
4	**Abiesin**	
5	**Gingerenone A**	
6	**Nerolidol**	

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
Beta Sitosterol	414.718 g/mol	$C_{29}H_{50}O$	1	1	6
Kaempferol	286.24 g/mol	$C_{15}H_{10}O_6$	4	6	1
Piperine	285.34 g/mol	$C_{17}H_{19}NO_3$	0	3	3
Abiesin	580.5 g/mol	$C_{33}H_{24}O_{10}$	3	10	6
Gingerenone A	356.4 g/mol	$C_{21}H_{24}O_5$	2	5	9
Nerolidol	222.37 g/mol	$C_{15}H_{26}O$	1	1	7

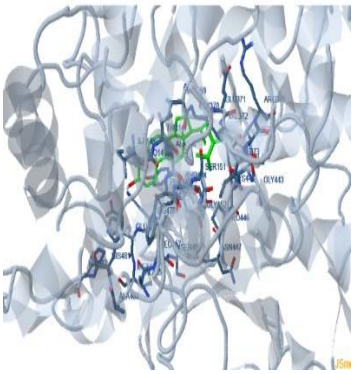
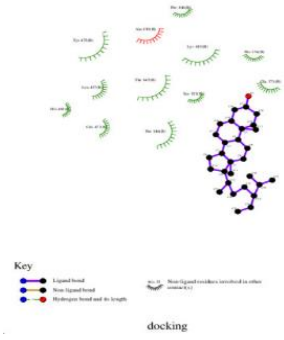
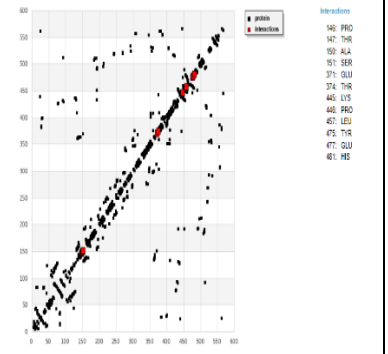
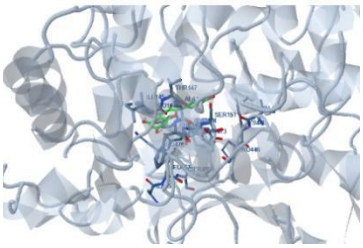
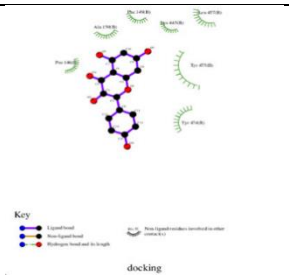
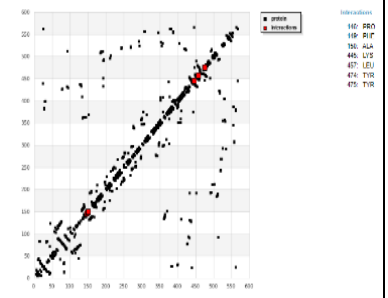
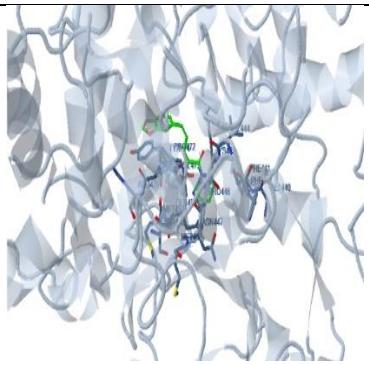
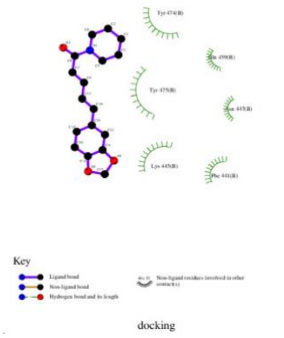
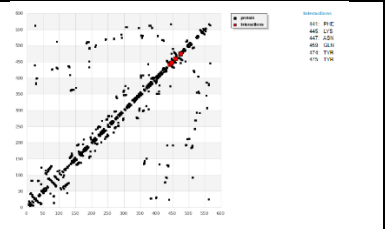
Table-3. Summary of the molecular docking studies of compounds against H pylori Urease with PDB 1E9Y

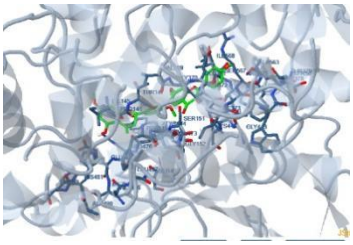
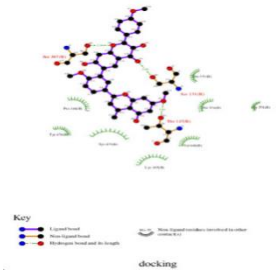
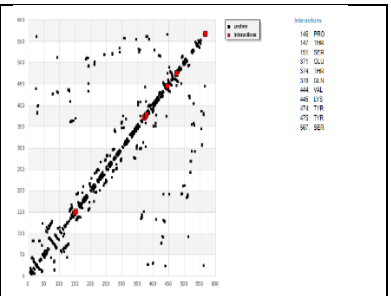
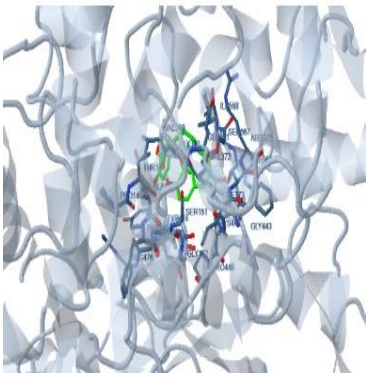
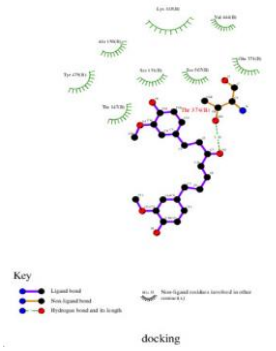
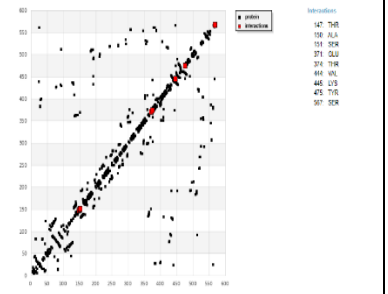
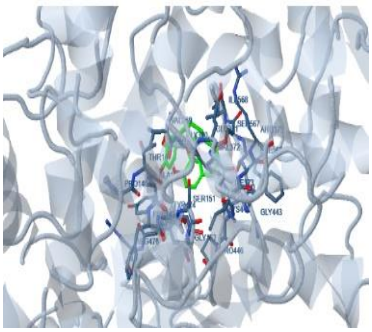
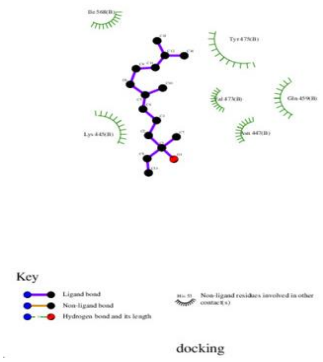
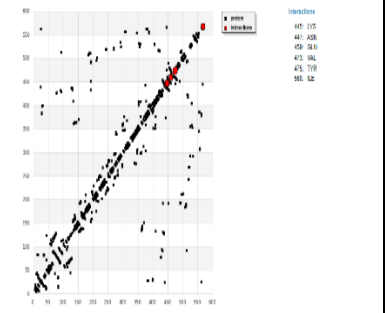
Compounds	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Beta Sitosterol	-8.10 kcal/mol	1.15 uM	-0.03 kcal/mol	-9.43 kcal/mol	793.756
Kaempferol	-5.00 kcal/mol	215.65 uM	-0.10 kcal/mol	-5.47 kcal/mol	556.873
Piperine	-6.42 kcal/mol	19.75 uM	-0.11 kcal/mol	-7.14 kcal/mol	591.711
Abiesin	-7.24 kcal/mol	4.90 uM	-0.58 kcal/mol	-8.86 kcal/mol	1080.93
Gingerenone A	-6.57 kcal/mol	15.36 uM	-0.05 kcal/mol	-5.34 kcal/mol	602.088
Nerolidol	-5.02 kcal/mol	209.70 uM	-0.09 kcal/mol	-7.18 kcal/mol	612.375

Table-4. Amino acid Residue Interaction of Lead compundsagainst H pylori Urease with PDB 1E9Y

Compound	Interactions	Amino acid residues											
β-sitosterol	2	146 PRO	147 THR	150 ALA	151 SER	371 GLU	374 THR	445 LYS	446 PRO	457 LEU	475 TYR	477 GLU	481 HIS
Kaempferol	2	146 PRO	149 PHE	150 ALA	445 LYS	457 LEU	474 TYR	475 TYR					
Piperine	2	441 PHE	445 LYS	447 ASN	459 GLN	474 TYR	475 TYR						
Abiesin	2	146 PRO	147 THR	151 SER	371 GLU	374 THR	378 GLN	444 VAL	445 LYS	474 TYR	475 TYR	567 SER	
Gingerenone-A	2	147 THR	150 ALA	151 SER	371 GLU	374 THR	444 VAL	445 LYS	475 TYR	567 SER			
Nerolidol	2	445 LYS	447 ASN	459 GLN	473 VAL	475 TYR	568 ILE						

Table -5. Docking pose of phytochemicals of ElathyChooranam with PDB1E9Y, 2D interaction plot and hydrogen bonding with core amino acid

Phytochemical	Docking pose with H pylori Urease PDB 1E9Y	2D interaction plot analysis	Hydrogen bond plotting with core amino acid Analysis
β-sitosterol			
Kaempferol			
Piperine			

Abiesin			
Gingerenone-A			
Nerolidol			

DISCUSSION

Total of 6 bioactive lead compounds were retrieved from the herbs present in the siddha formulation ElathyChooranam.From reported data the phytochemicals such as β-sitosterol , Kaempferol , Piperine, Abiesin, Gingerenone-A and Nerolidol reveals maximum of 3 interactions that accounts of 75% of the occupancy with the core active amino acid residues present on the target protein enzyme H pylori urease. Evidence from ulcerogenic animal model studies show that certain dietary phospholipids and phytosterols like β-sitosterol, stigmasterol, and isomers of β-sitosterol have a protective action against gastroduodenal ulceration, both singly and in combination. [22]

β-sitosterol with a molecular mass of 414.718 g/mol and 6 rotatable bonds (Table 2) showed remarkable binding affinity of -8.10 kcal/mol with an estimated Ki of 1.15 uM (Table 3) with the largest amino acid (12 aa) pocket (Table 4) and interacting with lys445 and ty475 of urease enzyme through this study.Through many pharmacologic studies, β-sitosterol has been deemed likely safe when taken orally in recommended doses for up to six months for BPH or for cholesterol-lowering effects. [23]

A previous study shows no inhibitory activity (IC50) for β-sitosterol and kaempferol against Canavalia ensiformisurease enzyme. [24]. In contrast, molecular docking studies confirm inhibitory activity of kaempferol against jack bean urease with Ki and binding energy equal 17.92 μM and − 6.48 kcal/mol. [25]

In this study, kaempferol with a molecular mass of 286.24 g/mol (Table 2) showed the least binding affinity of all the selected compounds, i.e., -5.00 kcal/mol with a highest estimated Ki of 215.65 uM (Table 3) and interacting with lys445 and ty475 of urease enzyme (Table 4).Studies have demonstrated that kaempferol can inhibit the activity of H. pylori urease, where it was found to significantly reduce the ulcer index in a rat model of H. pylori-induced gastritis [26]. Another study found that kaempferol was able to inhibit H. pylori growth and the production of urease [27]. Other studies have also reported similar findings, suggesting that kaempferol may be a promising candidate for the treatment of H. pylori-induced gastritis and ulcers

[28].

Piperine, a natural compound found abundant in Piper nigrum with a molecular mass of 285.34 g/mol (Table 2) and no H-bond donor showed moderate binding affinity of -6.42 kcal/mol with a highest estimated Ki of 19.75 uM (Table 3) and interacting with lys445 and ty475 of urease enzyme (Table 4).Piperine is already reported to have protective properties against gastric ulcers. [29]. It was observed that piperine inhibited H. pylori growth completely at a minimal inhibitory concentration of 125 μM and IC50 value of 115 μM. [30]

A study published found that piperine was able to inhibit the adherence of H. pylori to gastric cells and prevent toxin entry in to gastric epithelium consecutively decreasing the risk of oncogenesis. [31] [32]

Gingerenone A is a natural compound occurring in ginger (Zingiber officinale) extract which is reported to have inhibited the growth of Helicobacter pylori in vitro with a minimum inhibitory concentration in the range 0.78 to 12.5 μg/mL tested in a rodent model of H. pylori-induced disease. [33] Molecular docking studies of Gingerenone A with the highest number of rotatable bonds (Table 2) revealed a binding affinity of -6.57 kcal/mol (Table 3) and Ki of 15.36 uM against urease.

Nerolidol is reported to have cytoprotective and anti-ulcer properties. This mechanism by nerolidol is achieved through increased mucus production, gastric bicarbonate and gastric blood flow. [34] [28] Anti-Helicobacter pylori and antiulcerogenic activity of Aframomumpruinosum seeds rich in (E)-nerolidol was also reported with an MIC value of 128 μg/mL in methanol extract. [35]

Nerolidol with a molecular mass of 222.37 g/mol (Table 2) in our docking studies revealed a least -5.02 kcal/mol of binding affinity (Table 3). The binding affinity for Nerolidol is 209.70 uM and interacting with lys445 and ty475 of urease enzyme (Table 4).There is no previous report for abiesin's activity against urease till now. With a highest number of H-donors and molecular weight of 580.5 g/mol (Table 2), it has a good binding affinity of -7.24 kcal/mol and Ki of 4.90 uM (Table 3).

CONCLUSION

This study was aimed to identify bioactive phytochemicals that can inhibit target protein enzyme H pylori urease.Based on the results of the computational analysis it was concluded that the bio-active compound's like β-sitosterol , Kaempferol , Piperine, Abiesin, Gingerenone-A and Nerolidol present in the siddha formulation ElathyChooranam possess significant binding against the target H pylori urease by interacting with active amino acid present on the active site thereby it was concluded that these compounds may exerts promising anti-ulcer activity by inhibiting the enzyme H pylori urease that catalyze the hydrolysis of urea to ammonia. This confirms the reverse pharmacological evidence of the siddha formulation Elathy Chooranam to have anti H.Pylori activity and therefore can be administered to H.Pylori induced ulcers .

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