Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 12, Issue 4, 2023, 91
https://doi.org/10.33263/LIANBS124.091

Computational Approach Assessing the Antibacterial Activity of *Acorus calamus* Against *Helicobacter pylori*

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Scopus Author ID 54414904600
Received: 11.03.2022; Accepted: 18.04.2022; Published: 24.05.2022

Abstract: *Helicobacter pylori* is a gram-negative, microaerophilic bacteria that lives in the stomach of most of the human population. Overview of novel alternatives utilizing common natural phytochemicals has been demonstrated as powerful tools in bringing down the bacterial levels, managing inflammation, and regulating the immune response. Vacha (*Acorus calamus* Linn. (*Acoraceae*)) is a traditional Indian medicinal plant used to cure various illnesses. Our study strongly supports the therapeutic use of *Acorus calamus Linn*. phytocompounds as a possible herb that can be considered to inhibit *Helicobacter pylori*. This study aimed to identify the phytochemicals of the *Acorus calamus* that can inhibit *H.pylori*. Here we are using *in silico* molecular docking approach by using bioinformatics tools. In conclusion, our study strongly supports the medicinal use of *Acorus calamus* Linn. phytocompounds as a possible herb that can be considered to inhibit *Helicobacter pylori*. Further research may be performed on the phytocompounds, shyobunone, and 2,3,5-trimethoxyamphetamine to determine the exact action mechanism and validate the obtained results further.

Keywords: molecular docking; *Acorus calamus* Linn; *Helicobacter pylori*; phytocompounds.

1. Introduction

Helicobacter pylori is a gram-negative, microaerophilic bacteria that lives in the stomach of the majority of the human population. This bacterium, which has a global distribution, is known to cause illnesses that impact at least half of the world's population. It possesses a characteristic helical shape, about 3 micrometers long with a diameter of about 0.5 micrometers [1,2]. All helicobacters are motile with 4-6 flagella and a slower wave-like motion due to flagellar activity. Electron dense granules are observed. These bodies are polyphosphate

aggregates and serve as a reserve source of energy. It is microaerophilic, requiring carbon dioxide and reduced oxygen concentration for growth. It raises the gastric pH by converting urea to ammonia and carbon dioxide [2,3].

Helicobacter pylori (*H.pylori*) can enter a body and live in the digestive tract in the upper part of the small intestine. *H.pylori can be transmitted from person to person through direct contact with saliva and faeces, as well as contaminated food and water*. [3].

Symptoms: burning pain in the abdomen, nausea, loss of appetite, bloating, unintentional weight loss.

The essential enzyme generated by *H.pylori* is urease, which allows the bacterium to survive in low pH environments and helps colonization of the stomach mucous membrane. The enzyme is responsible for urea degradation to ammonia and carbon dioxide. The leading cause of ulcers is when the bacteria attack the lining that protects the stomach and make an enzyme called urease [3]. *H.pylori* strains have a high molecular mass protein encoded by cagA and vacuolating toxin encoded by vacA. CagA and vacA, both secreted proteins, have been postulated to be more virulence factors in Helicobacter. CagA is injected into cells by bacterial type IV secretions apparatus where it phosphorylates host cell signaling proteins causing numerous changes in host cell gene expression. Vacuolating cytotoxin, VacA forms pores in host cell membranes, damaged mitochondria, and decreases endothelial nitric oxide production [4].

Currently, there are several diagnostic approaches for detecting the presence of this infection, each with its own set of benefits, drawbacks, and limitations. On tissue obtained by endoscopy, biopsy-based procedures such as polymerase chain reaction (PCR), histological examination, culture, and the fast urease test (cod liver oil test/CLO 2 test) are performed [3]. On the other hand, non-invasive methods include the urea breath test (C13-C14), serology to find IgG antibodies, and stool antigen test (SAT). The treatment required by the patient is decided based on the diagnosis of the *H.pylori* infection and the clinical status. Antimicrobials and gastric acid suppression have traditionally been treated [4]. Several combinations of drugs, such as triple regimens, are utilized to manage and remove the infection. The medications of choice include clarithromycin, tetracycline, amoxicillin, bismuth salts, and metronidazole/tinidazole with omeprazole/lansoprazole/pantoprazole (proton pump inhibitors) [4,5]. However, these treatments can be of significant risk because of resistance to antibiotics and related adverse impacts. Overview of novel alternatives utilizing common natural phytochemicals has been demonstrated as powerful tools in bringing down the bacterial levels, managing inflammation, and regulating the immune response.

Vacha (*Acorus calamus* Linn. (Acoraceae)) is an Indian traditional medicinal plant used to cure various neurological, gastrointestinal, pulmonary, metabolic, renal, and liver problems. The plant's rhizomes are brown in color, twisted, cylindrical, curled, and have a short nod [6]. The leaves have a thick sword-like shape in the center and have curved borders. Sterols, Phenylpropanoids, triterpenoid saponins, triterpene glycosides, sesquiterpenoids, monoterpenes, and alkaloids have been identified from *A.calamus* rhizomes and left too far [7]. Phenylpropanoids (most notably asarone and eugenol) and sesquiterpenoids have been identified as the most potent chemicals in *A.calamus*. α-Asarone and β-Asarone have been reported to possess antimicrobial properties against several microbial species. The three main compounds with antibacterial, anti-inflammatory, and antioxidant properties are alkaloids, palmitic, and linoleic acids. In the rhizome extract, shyobunone is a keto molecule, whereas α-Asarone and β-Asarone are aromatic compounds [8,9]. Even though *A.calamus* roots,

rhizomes, and essential oils possess several beneficial biological activities, sufficiently not enough effort has been devoted to studying their antibacterial properties [10]. This study aimed to identify the phytochemicals of the *Acorus calamus* that can inhibit *H.pylori*. Here we are using *in silico* molecular docking approach by using bioinformatics tools. Docking enables quick virtual screening of approved drugs, natural products, or already synthesized compounds against one or more biological targets of interest. The goal of molecular docking is to use computational methods to estimate the structure of the ligand-receptor complex. Docking is accomplished in two steps: first, sampling ligand conformations in the protein's active site, and then ranking these conformations using a scoring function [11,12].

2. Materials and Methods

2.1. Protein preparation and validation.

Based on the review of literature, LuxS and ToxB proteins of H .pylori were selected for the docking analysis. The three-dimensional structures of these proteins were retrieved from the protein data bank (PDB) (Figures 1 & 2). These structures were then validated using UCLA-DOE LAB — SAVES v6.0 web server. The PROCHECK program of this server was run to determine the percentage of residues in the allowed region [13].

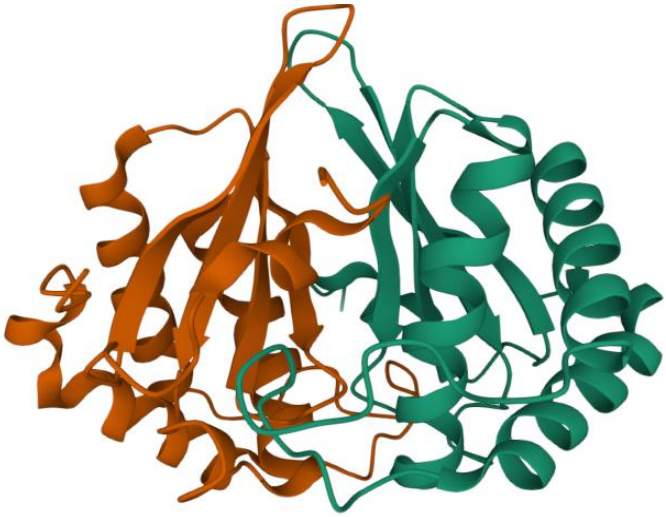


Figure 1. Ribbon structure of *Helicobacter pylori* LUXS (PDB ID: 1J6X) [14].

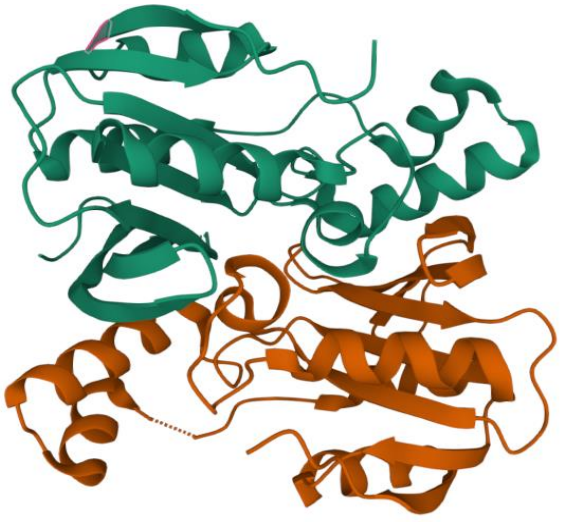


Figure 2. Crystal structure of *Helicobacter pylori* TOXB represented in ribbon format (PDB ID: 4RL4) [15].

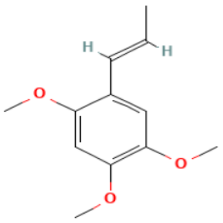
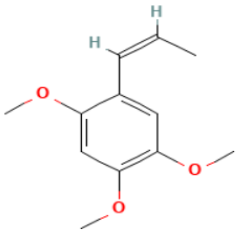
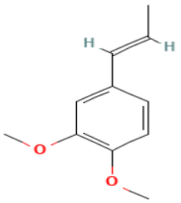
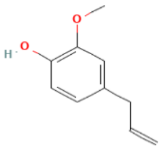
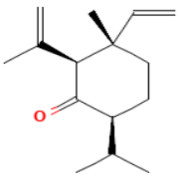
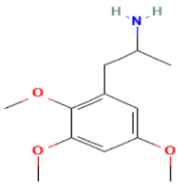
2.2. Binding site prediction.

Computed Atlas of Surface Topography of proteins (CASTp) 3.0 webserver was used to determine the binding residues of the target proteins. It identifies pockets and cavities analytically. It also determines the area and volume of the pocket [16].

2.3. Ligand preparation.

The principle effective compounds that have been proven to show numerous biological activities have been selected as the ligands to inhibit the target proteins of *H.pylori*. Table 1 consists of the ligands along with their 2D structures. The 3D structures of these ligands were retrieved from the PubChem database [17].

Table 1. Two-dimensional structures of the principle phytocompounds present in *A.calamus*

Phytocompounds of *A.calamus*	**2D Structures**
alpha-Asarone (CID: 636822)	
beta-Asarone (CID: 5281758)	
Methyl isoeugenol (CID: 637776)	
Eugenol (CID: 3314)	
Shyobunone (CID: 5321293)	
2,3,5-trimethoxyamphetamine (CID: 602804)	

2.4. Molecular docking.

The protein structures were prepared for molecular docking by removing the co-existing ligands. Water molecules were removed, Gasteiger charges were added, and missing residues were checked, repaired, and converted to pdbqt format. The downloaded 3D structures were converted from sdf format to PDB format, the required file format for docking. These prepared files were loaded to the PyRx 0.8v workspace. PyRx is a virtual screening tool to carry out molecular docking analysis. The binding residues determined by CASTp software were selected, and a grid box was generated surrounding the proteins for the ligands to bind [18,19].

3. Results and Discussion

The PROCHECK program was used to validate the three three-dimensional structures of *H.pylori* target proteins. According to the data, for both the protein IDs 4RL4 and 1J6X, 90.0% and 95.5% of residues were in the most favored area (Figure 3).To analyze the results of molecular docking, binding affinity (Kcal/mol), hydrogen bonding, and other non-bonded interactions are considered. Lowering the binding affinity value better is the docking result. Various non-bonded interactions are formed, such as van der Waals, pi-pi, n-Alkyl, pi-sigma, etc.

CASTp 3.0 computed a single binding pocket for each protein. For the *Helicobacter pylori* LUXS protein, a pocket of an area (SA) of 856.525 and a volume (SA) of 467.286 was identified (Figure 4). This protein was predicted to have 40 amino acid residue at the active site. For the *Helicobacter pylori* TOXB protein, a pocket of an area (SA) of 453.953 and a volume (SA) of 376.827 were identified with a total of 35 amino acid residue at the active site (Figure 5). Moreover, the more favorable sites were selected for the docking analyses.

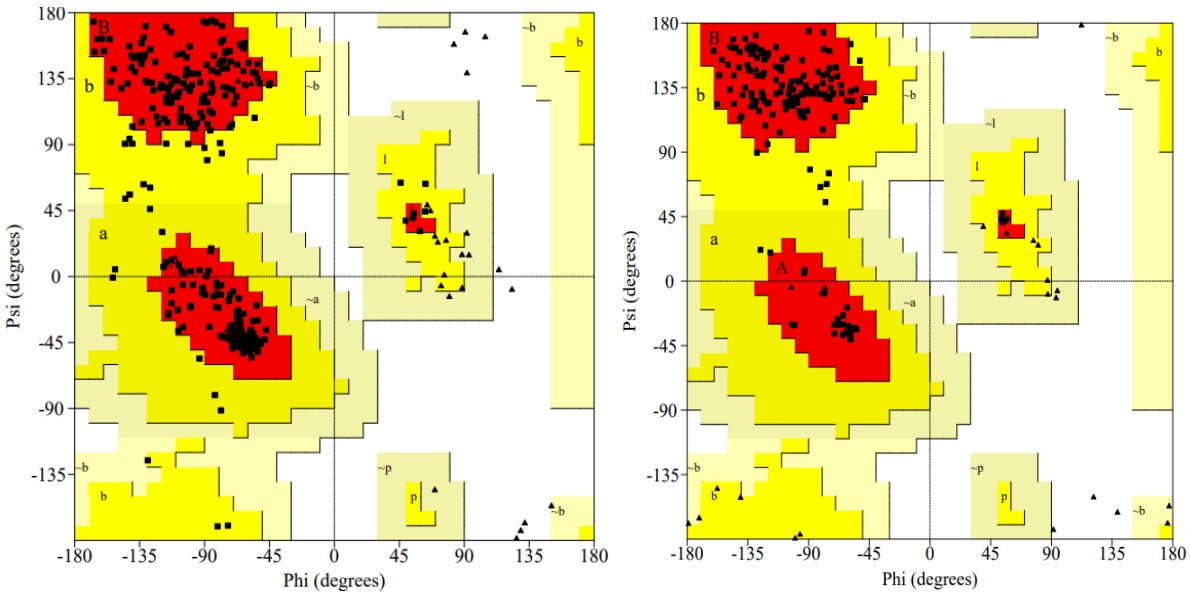


Figure 3. Ramachandran plot validation of three-dimensional structures of proteins a) 4RL4 and b) 1J6X.

Figure 6 represents the grid box surrounding the binding site residues selected in the PyRx virtual screening tool workspace. The grid box for the LUXS protein had dimensions of x=39.30, y=39.95, z=35.71, and for that of TOXB protein, the values were x=33.38, y=29.02, z=40.88.

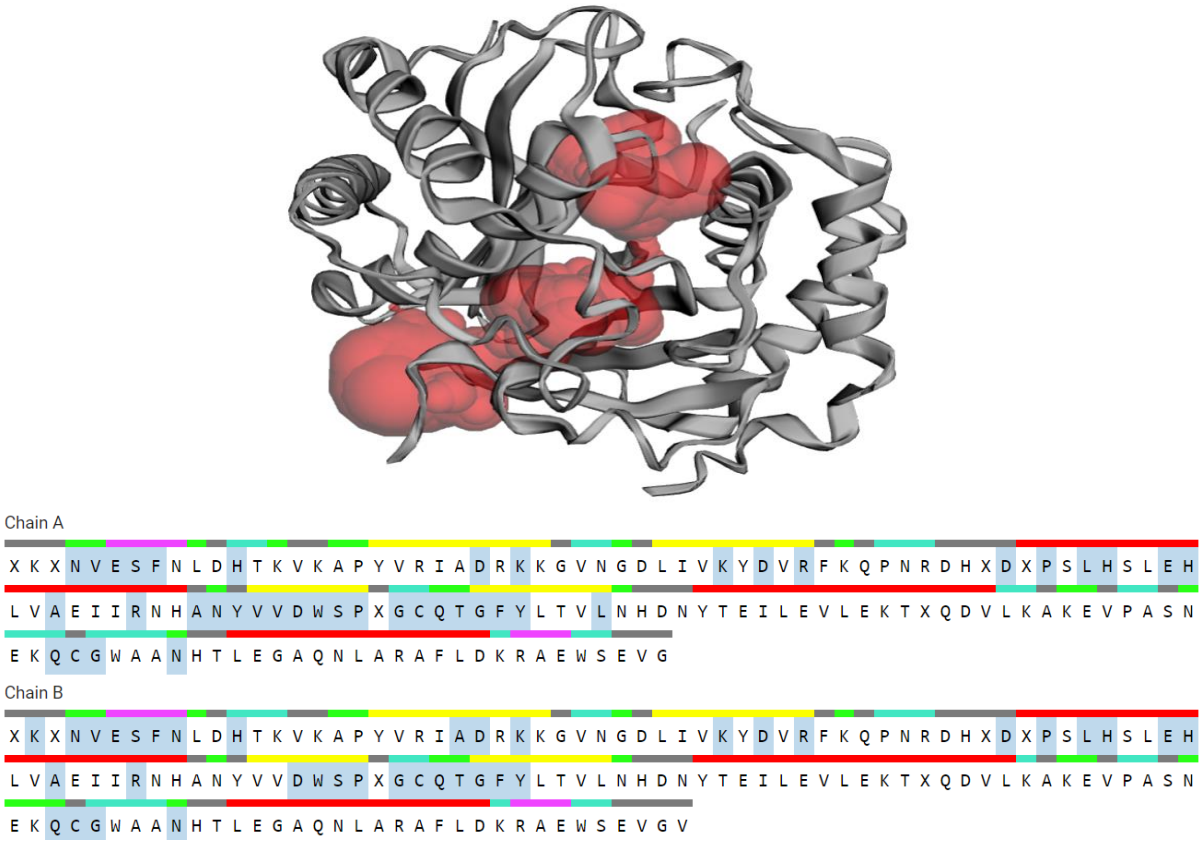


Figure 4. Representation of binding pocket (red) and the highlighted residues of the *Helicobacter pylori* LUXS protein forming the binding site as computed using CASTp 3.0v.

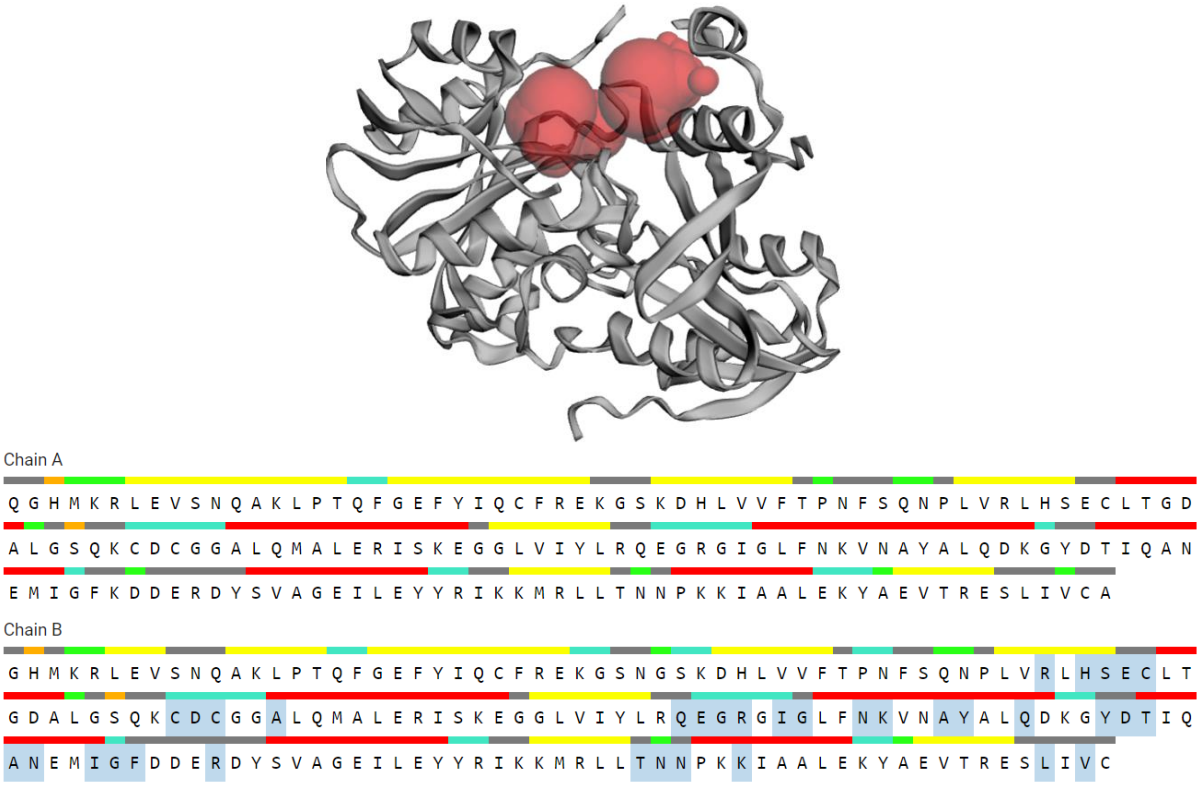
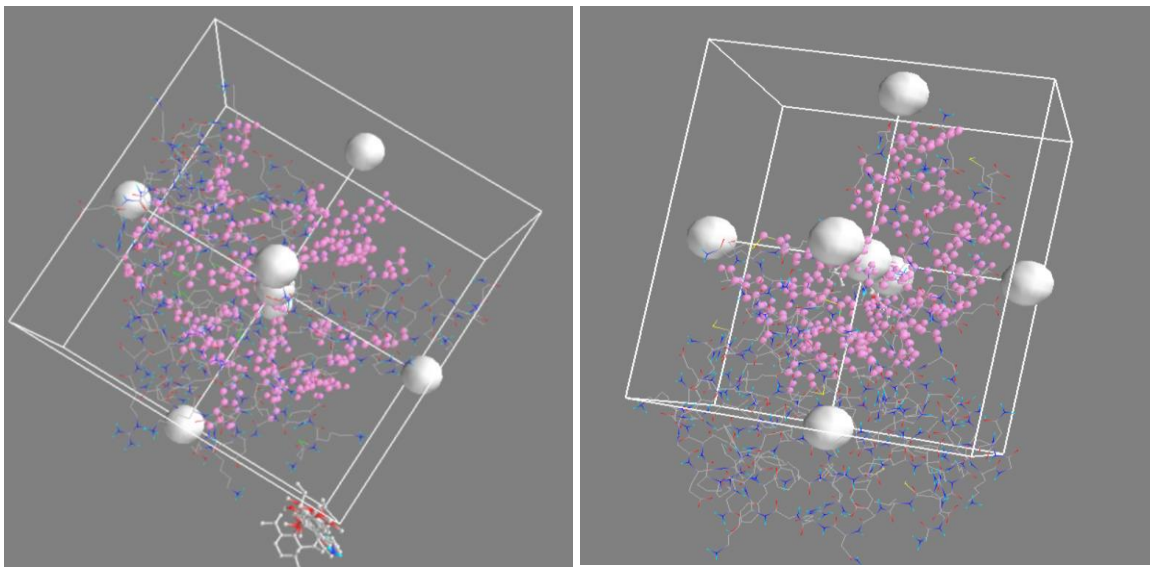


Figure 5. Representation of binding pocket (red) and the highlighted residues of the *Helicobacter pylori* TOXB protein forming the binding site as computed using CASTp 3.0v.

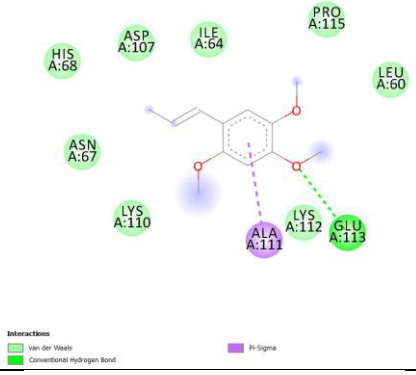
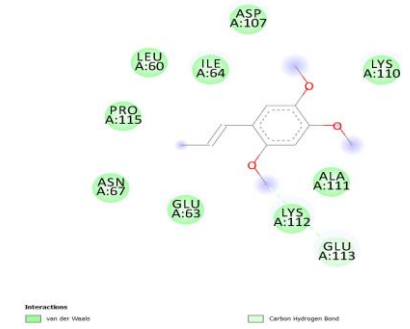


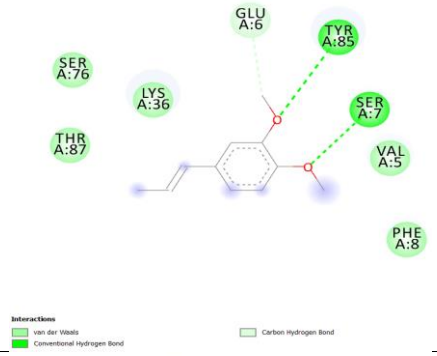
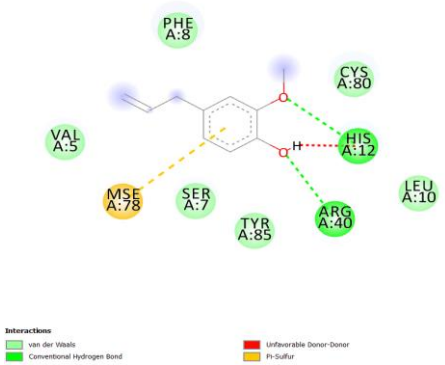
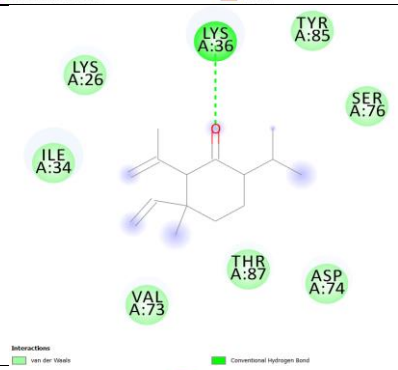
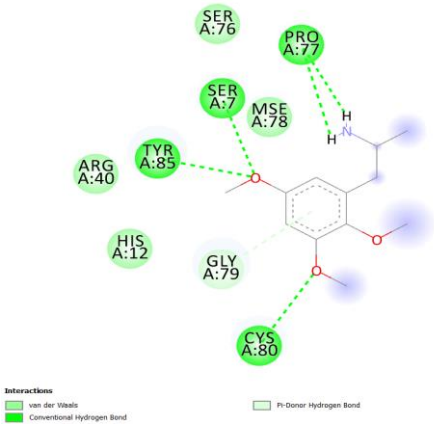
(**a**) (**b**)

Figure 6. The binding site residues (pink colored) surrounded by a grid box for the ligands to bind the target protein A) LUXS B) TOXB.

Molecular docking interaction studies are conducted to check the interactions of ligands with the selected *H.pylori* proteins. PyRx software determines nine poses of the docked ligand with a protein. Out of the nine poses, a single pose with the least binding energy and highest number of interactions is chosen and visualized using BIOVIA Discovery Studio visualizer.

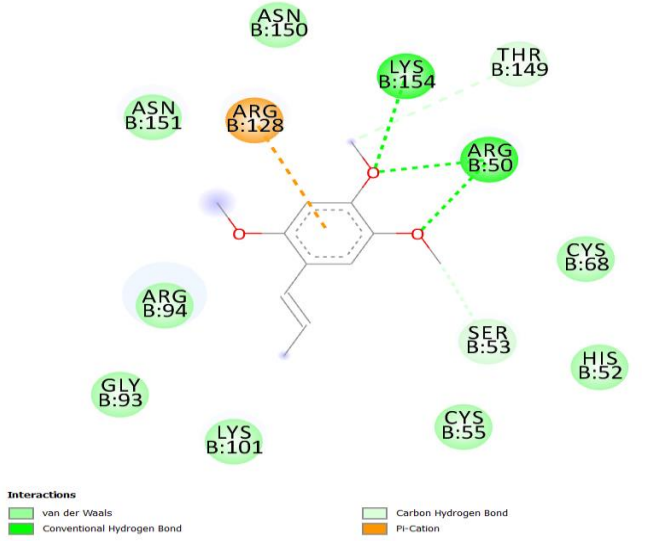
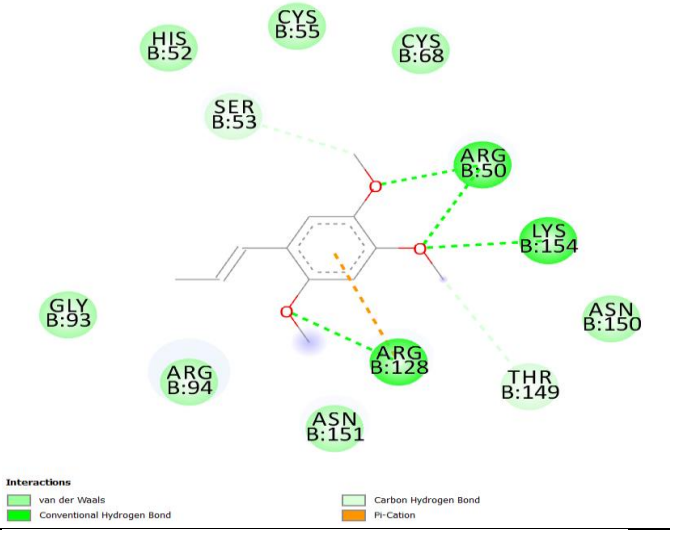
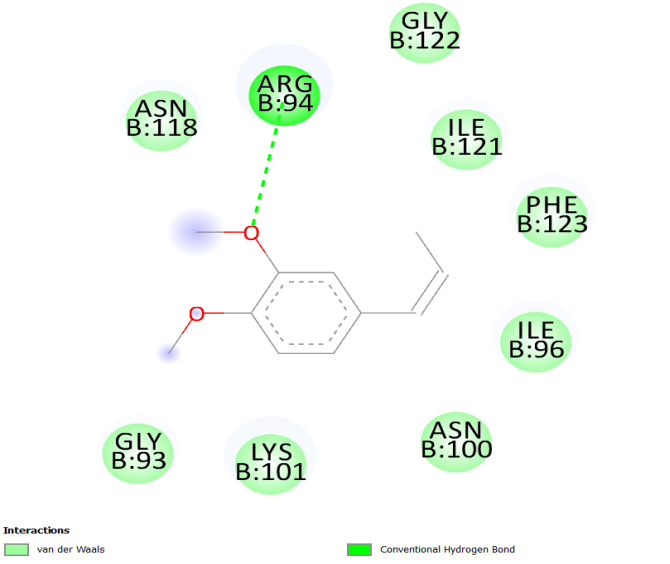
Table 2. Molecular docking results of *Helicobacter pylori* LUXS protein with phytocompounds of *A.calamus* determine the binding affinities and amino acid residues forming the hydrogen bonds.

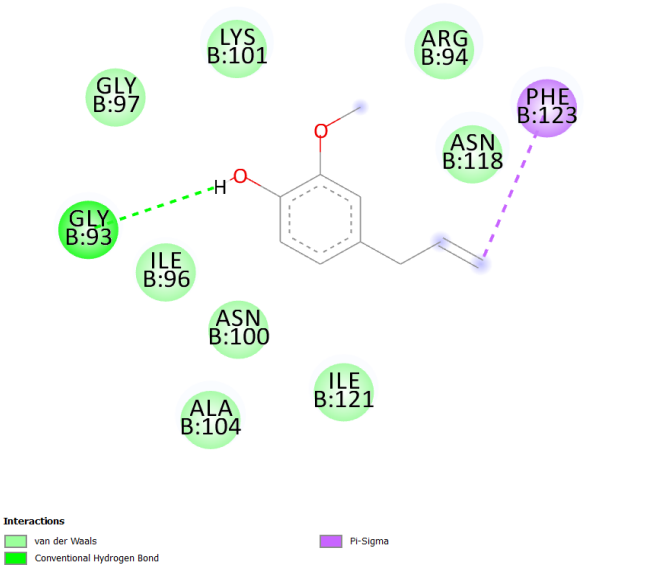
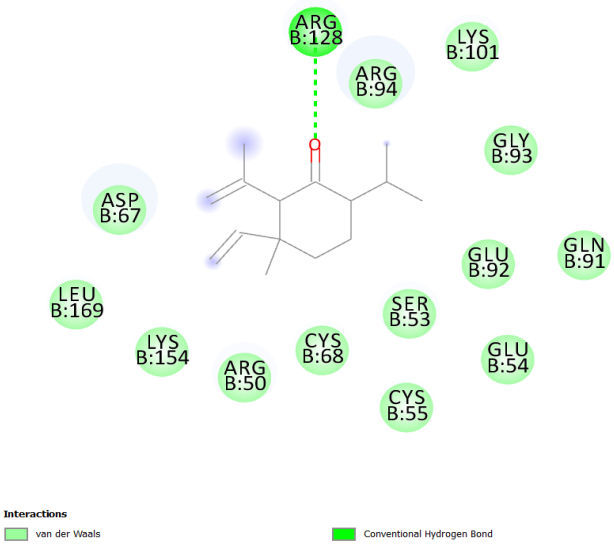
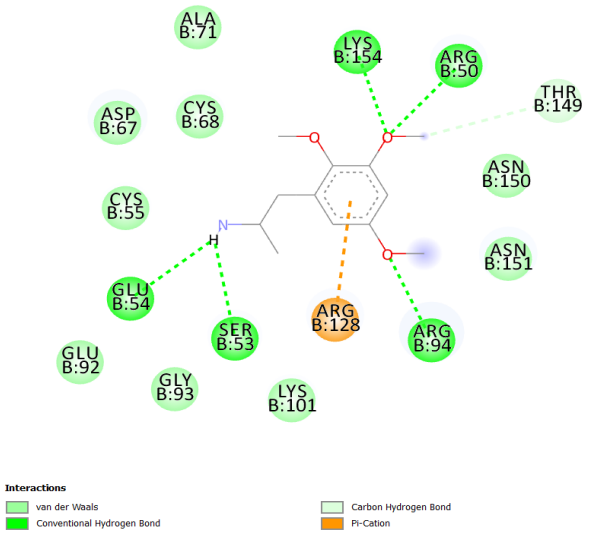
Phytocompounds	**Binding affinity (Kcal/mol)**	**Hydrogen bonds**	**2D representation of the intermolecular non-bonded interactions between binding site residues of the protein and ligand**
alpha-Asarone	-4.6	GLU A:113	
beta-Asarone	-4.7	NIL	

Phytocompounds	Binding affinity (Kcal/mol)	Hydrogen bonds	2D representation of the intermolecular non-bonded interactions between binding site residues of the protein and ligand
Methylisoeugenol	-4.6	SER A:7, TYR A:85	
Eugenol	-4.7	HIS A:12, ARG A:40	
Shyobunone	-6.3	LYS A:36	
2,3,5-trimethoxyamphetamine	-5.7	SER A:7, SER A:76, PRO A:77, CYS A:80, TYR A:85,	

Based on the above molecular docking results of *Helicobacter pylori* LUXS protein (Table 2) with phytocompounds of *A.calamus*, Shyobunone showed the least binding energy of -6.3 Kcal/mol forming a single hydrogen bond with LYS A:36 and var der Waals interactions with LYS A:26, ILE A:34, VAL A:73, ASP A:74, SER A:76, TYR A:85, THR A:87. 2,3,5-trimethoxyamphetamine displayed the highest interactions with a binding affinity of -5.7 Kcal/mol, forming a hydrogen bond each with SER A:7 and TYR A:85 and two hydrogen bonds with PRO A:77, van der Waals interactions with HIS A:12, ARG A:40, SER A:76, and a pi-donor hydrogen bond GLY A:79.

Table 3. Molecular docking results of *Helicobacter pylori* TOXB protein with phytocompounds of *A.calamus* determining the binding affinities and amino acid residues forming the hydrogen bonds.

Phytocompounds	Binding affinity (Kcal/mol)	Hydrogen bonds	2D representation of the intermolecular non-bonded interactions between binding site residues of the protein and ligand
alpha-Asarone	-5.6	ARG B:50, LYS B:154	
beta-Asarone	-6	ARG B:50, ARG B:128, LYS B:154	
Methylisoeugenol	-5.6	ARG B:94	

Phytocompounds	Binding affinity (Kcal/mol)	Hydrogen bonds	2D representation of the intermolecular non-bonded interactions between binding site residues of the protein and ligand
Eugenol	-6.2	GLY B:93	
Shyobunone	-6.5	ARG B:128	
2,3,5-trimethoxyamphetamine	-6.3	ARG B:50, SER B:53, GLU B:54, ARG B:94, LYS B:154	

The molecular docking results of *Helicobacter pylori* TOXB protein (Table 3) with phytocompounds of *A.calamus*, Shyobunone and 2,3,5-trimethoxyamphetamine showed the

least binding energy with -6.5 Kcal/mol and -6.3 Kcal/mol respectively in comparison with other ligands. As represented in the 2D diagram in table 3, Shyobunone forms a hydrogen bond with ARG B:128 and several van der Waals interactions with the residues ASP B:67, LEU B:169, LYS B:154, ARG B:50. CYS B:68, SER B:53, CYS B:55, GLU B:54, GLN B:91, GLU B:92, GLY B:93, ARG B:94, and LYS B:101. 2,3,5-trimethoxyamphetamine can be seen forming the highest hydrogen bonds with ARG B:50, SER B:53, GLU B:54, ARG B:94, and LYS B:154 and other non-bonded interactions like carbon-hydrogen bond with THR B:149, pi-cation with ARG B:128 and van der Waals with CYS B:55, ASP B:97, CYS B:68, ALA B:71, GLU B:92, GLY B:93, LYS B:101, ASN B:150, and ASN B:151.

4. Conclusions

Through various mechanisms, *A. calamus* greatly affects fasting blood sugar, insulin resistance, HbA1c, and the adipogenic transcription expression factor, including antioxidant, anti-inflammatory, cell regeneration, improved insulin sensitivity, gluconeogenesis, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and GLUT-4-mediated transport inhibition. It is microaerophilic and thrives on carbon dioxide and low oxygen levels. It uses the enzyme urease to convert urea to ammonia and carbon dioxide, elevating the upper intestine's stomach pH, which is generally 46.5. We know from this work that 2,3,5-trimethoxyamphetamine is a strong phytochemical in *A. calamus*, and that it can be tested *in vitro*, making it a unique therapeutic option for *H.pylori* treatment.

The purpose of the present study was to provide insight into how the phytoconstituents present in a plant help inhibit the main target proteins of *H.pylori*. In conclusion, our study strongly supports the medicinal use of *Acorus calamus* Linn. phytocompounds as a possible herb that can be considered to inhibit *Helicobacter pylori*. Further research may be performed on the phytocompounds, shyobunone, and 2,3,5-trimethoxyamphetamine to determine the exact action mechanism and validate the obtained results further. Other *in silico* analyses like molecular dynamics simulations and free energy perturbation (FEP) calculations can be performed on these docked complexes to understand better the stability and interactions between the target protein and the phytocompound. These compounds can be considered additionally for *in vitro* evaluation, making them a novel therapeutic alternative to treating *H.pylori*.

Funding

This research received no external funding.

Acknowledgments

The authors thank the Director and Head, JSS Academy of Higher Education and Research (JSSAHER), Mysuru, India, for the support and infrastructure facilities. SPK is grateful to the Director, Amrita Vishwa Vidyapeetham, Mysuru campus, for infrastructure support. The authors acknowledge the Director, Davangere University, Davangere, for the computational lab facilities.

Conflicts of Interest

The authors declare no conflict of interest.

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