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In-Silico investigation of Anti-ulcer potential of the herbal leads against *Helicobacter pylori* urease

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Abstract

Around four million people on the face of the earth are diagnosed with gastric ulcers every year. Consuming alcohol, smoking cigarettes, having an infection caused by *Helicobacter pylori*, or taking non-steroidal anti-inflammatory medicines (NSAIDs) can all contribute to the development of this diverse disease with a complex etiology. This illness affects somewhere between 10 and 15 percent of the total population around the globe. Production of urease is the most crucial virulence factor mediated by *H.pylori* infection. Conventional anti-ulcer agents are known for exerting potential side effects such as gynecomastia, impotence, osteoporotic bone fractures, deficiencies of iron and magnesium etc. Medicinal plants have been utilised in medicine since the dawn of mankind. Global studies have been conducted to validate their efficacy, and some of the findings have resulted in the manufacture of plant-based medications. The main aim of the present investigation is to explore the anti-ulcer potential of the phytocomponents such as Berberine, Ellagic acid, Gallic acid, Huperzine A, Nicotinic acid, Palmatine, Pyridoxine, Rivastigmine and Spathulenol against the enzyme against *helicobacter pylori* ureaseby using AutoDock prediction. Results of the present investigation reveals that the compounds such as Pyridoxine, Berberine, Ellagic acid, Gallic acid, Huperzine A, Nicotinic acid, Palmatine, Rivastigmine and Spathulenol reveals significant binding affinity with that of the target amino acid residues present over the target enzyme *Helicobacter pylori* urease. It was concluded from the datas of the present study that the phytotherapeutics present in the herbs possess significant anti-ulcer activity and may be recommended for the clinical management of the same in near future with prior clinical justification.

Keywords: Ulcer, *Helicobacter pylori*¸ Urease, Phytocomponents, Docking, Auto-Dock,Anti-ulcer activity.

1. Introduction

Peptic ulcer disease (PUD) caused by inadequate mucosal defences and an overproduction of stomach acid-pepsin. An estimated 4,000,000 persons are afflicted by it every year [1]. PUD is thought to affect anywhere from 1.5% to 3% of the population annually [2]. The yearly incidence rates of PUD were estimated to be 0.10%-0.19% for PUD diagnosed by physicians and 0.03%-0.17% based on hospitalization data [3] after a meta-analysis of seven studies from developed nations. Acute sickness is caused by ulcer perforation in about 2%-14% of PUD patients [4,5]. Patients with perforated peptic ulcer (PPU) commonly arrive with acute abdomen, which is associated with a significant risk for morbidity and death [6]. Perforation is a major consequence of PUD.

The ability of *H. pylori* to thrive in the unfavorable acidic environment of the stomach depends on the generation of large amounts (10–15% of total protein) of the enzyme urease [6]. *H. pylori* is able to thrive in the unfavorable acidic environment of the stomach because it generates large amounts of ammonia. Urease reduces the amount of acid produced by the host's stomach by turning urea into ammonia, which in turn makes it possible for *Helicobacter* to multiply. Interestingly, urease contributes to the pathogenicity of several other important human infections in addition to promoting mucosal inflammation. These infections include *Mycobacterium tuberculosis*, *Cryptococcus neoformans* (associated with lung infections), and *Proteus* spp. (associated with urinary tract infections) [7]. Urease also promotes mucosal inflammation.

Inhibition of the urease enzyme is a reasonable point of attack for eradicating infections caused by *H. pylori* colonies and, as a result, preventing the illnesses that are caused by these colonies [8]. The advent of antibiotic-resistant bacteria makes it absolutely necessary to come up with new treatments for this kind of sickness. In point of fact, the traditional "triple therapy" consisting of two antibiotics in addition to a proton pump inhibitor has been expanded to include bismuth compounds, making it the "quadruple therapy" in the fight against bacteria that are resistant to multiple antibiotics [9].

Acid suppression pharmaceuticals, such as type-2 histamine receptor antagonists and proton pump inhibitors, are the conventional therapies for gastric ulcers [10]. However, these medications can cause a variety of unpleasant side effects in some patients in expanding the risk of developing gynecomastia, impotence, osteoporotic bone fractures, deficiencies of iron and magnesium, and vitamin B12 hypergastrinemia when you stop taking them [11-12], there is a demand for medications that may be administered over the long term with a minimum of adverse effects. At the moment, the primary treatment for gastric ulcers is to inhibit the production of stomach acid. Peptic ulcers were traditionally treated with a variety of medicinal herbs, such as those from the mint family. Plants and phytomedicines exert their effects via a variety of different mechanisms, such as antioxidant, cyto-protective, or anti-secretory properties [13]. Antiulcer action can typically be observed in plant species that are rich in active components such as flavonoids, tannins, or terpenoids [14].Molecular dynamics (MD) is widely used as a powerful simulation method in many fields of molecular modelling. In the context of docking the main objective of the present investigation is to screen the anti-ulcer potential of the viable herbal leads and its enzyme inhibition potential against *helicobacter pylori* urease.

2. Materials and Methods

2.1. Protein-ligand docking

Computational molecular investigation was performed using Auto Dock version 4 which predicts interaction binding affinity between selected therapeutic lead with that of the protein target *helicobacter pylori* urease-PDB- 1E9Y

2.2. Protein preparation

Three dimensional (3D) structure of *helicobacter pylori* urease with protein data bank (PDB) - 1E9Y (Figure 1) retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) [15].

2.3. Ligand model preparation

Structures of the phytocomponents such as Berberine, Ellagic acid, Gallic acid, Huperzine A, Nicotinic acid, Palmatine, Pyridoxine, Rivastigmine and Spathulenol subjected to docking investigation were outlined using ChemDraw sketch software and converted from two dimension (2D) to3D structures. Figure 2 summarizing 2D and 3D structure of approved ligand subjected to molecular docking Investigation against helicobacter pylori urease with protein data bank (PDB) - 1E9Y.

2.4. Docking simulations

Molecular docking analysis were performed using licensed version of Auto Dock 4, which predicts interactions between FDA approved drug molecules with that of the selected protein target (*helicobacter pylori* urease) with protein data bank (PDB)- 1E9Y retrieved from Research Collaboratory for Structural Bioinformatics (RCSB). 3D componential structure of lead molecules and protein were docked using AutoDock analytical tool version 4. Docking simulations were performed using the programmed algorithm inbuilt with pre automation in the software. 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 [16-17].

3. Results

Docking score implicates the binding affinity between the lead and target higher the negativity in the value that showcase the level of potency of the drug. Development and advancement in the field of computational analysis increased the precision level in identifying the potential drug molecule and deriving its mechanism of action at target site. Selective alterations in the functional groups greatly minimize the non-specific binding and impedes the adverse event at clinical level. Total of 9 bioactive lead compounds were subjected to the docking screening. Out of nine compounds' the lead pyridoxine has maximum of 3 interactions accounts for 100% docking efficiency with the core active amino acid residues present on the target. Followed by this the compounds such as Berberine, Ellagic acid, Gallic acid, Huperzine A, Nicotinic acid, Palmatine, Rivastigmine and Spathulenol ranked second and with the maximum of 2 interactions with the active site of the target *Helicobacter pylori* urease. As shown in Table 2 and 3.

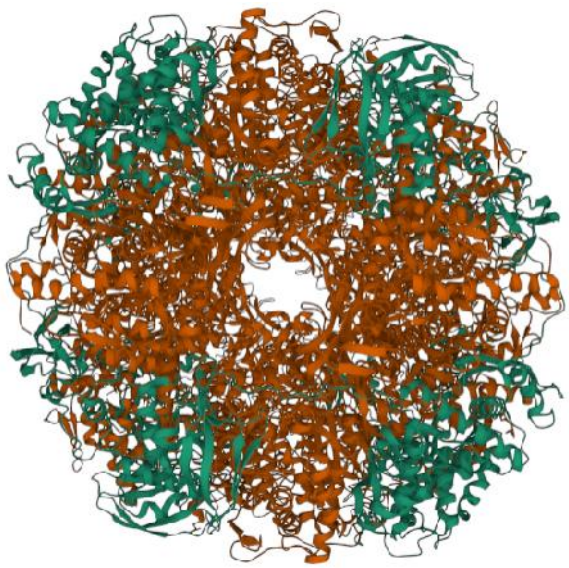

Figure 1: 3D crystalline structure of the target protein *Helicobacter pylori* urease – PDB 1E9Y

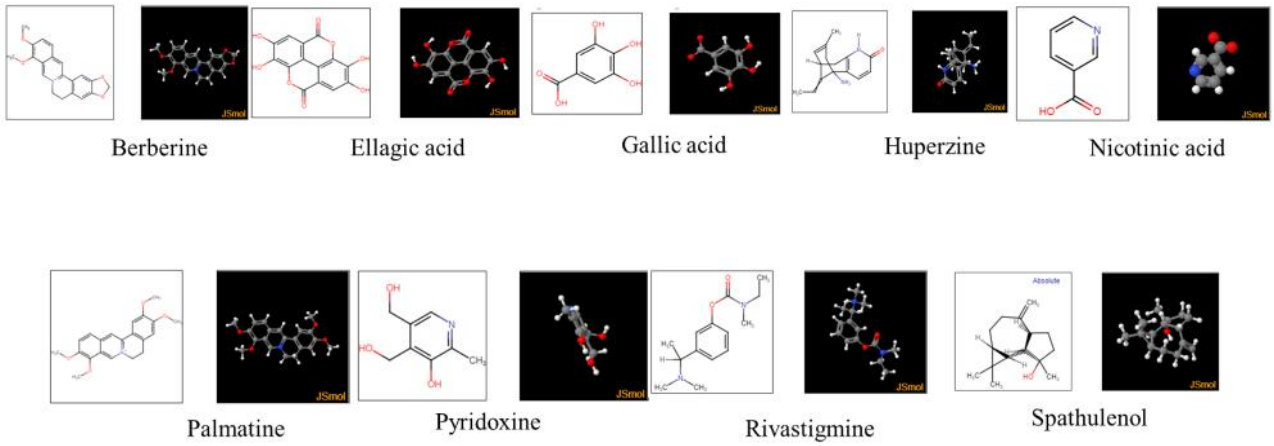

Figure 2: 2D and 3D Structure of Selected Ligands

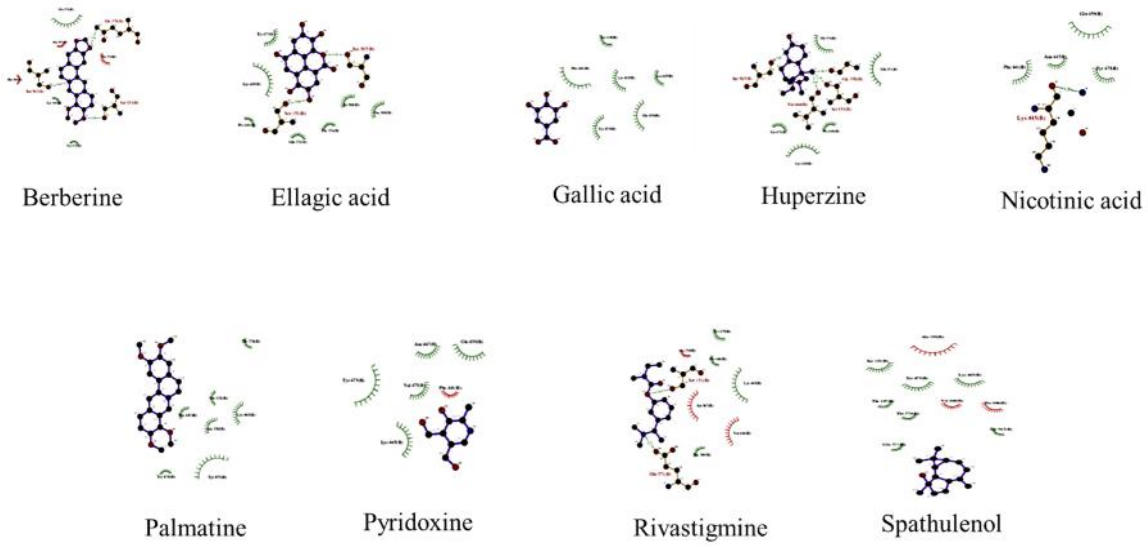

Figure 3: 2D Interaction plot of selected ligands with protein *Helicobacter pylori* urease – PDB 1E9Y

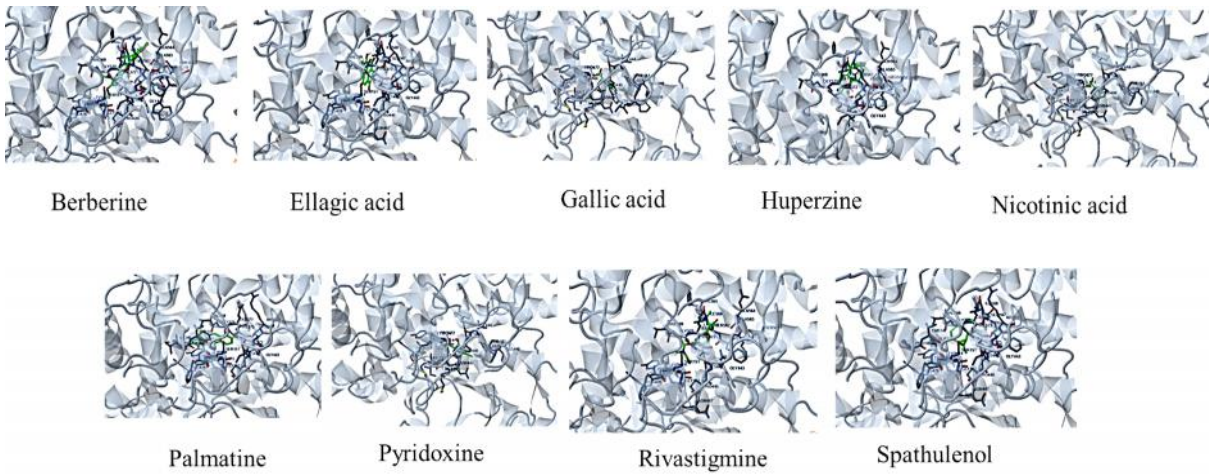

Figure 4: Docking pose of selected ligands with protein *Helicobacter pylori* urease – PDB 1E9Y

Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Berberine	336.4 g/mol	$C_{20}H_{18}NO_4$	3	0	4
Ellagic acid	302.194 g/mol	$C_{14}H_6O_8$	4	8	0
Gallic acid	170.12g/mol	$C_7H_6O_5$	4	5	1
Huperzine A	242.32 g/mol	$C_{15}H_{18}N_2O$	2	2	0
Nicotinic acid	123.11 g/mol	$C_6H_5NO_2$	1	3	1
Palmatine	352.4g/mol	$C_{21}H_{22}NO_4^+$	0	4	4
Pyridoxine	169.18 g/mol	$C_8H_{11}NO_3$	3	4	2
Rivastigmine	250.34 g/mol	$C_{14}H_{22}N_2O_2$	0	3	5
Spathulenol	220.35 g/mol	$C_{15}H_{24}O$	1	1	0

Table 2: Summary of the molecular docking studies of compounds against *Helicobacter pylori* urease – PDB 1E9Y

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Berberine	-5.81 kcal/mol	54.85 uM	-0.95 kcal/mol	-6.29 kcal/mol	**Interact. Surface**
Ellagic acid	-5.95 kcal/mol	43.57 uM	-0.05 kcal/mol	-4.94 kcal/mol	722.932
Gallic acid	-5.34 kcal/mol	122.49 uM	-0.38 kcal/mol	-4.88 kcal/mol	535.852
Huperzine A	-7.30 kcal/mol	4.45 uM	-2.03 kcal/mol	-7.60 kcal/mol	412.434
Nicotinic acid	-3.93 kcal/mol	1.33 mM	-0.12 kcal/mol	-4.22 kcal/mol	596.485
Palmatine	-4.96 kcal/mol	229.73 uM	-0.45 kcal/mol	-6.05 kcal/mol	337.794
Pyridoxine	-3.87 kcal/mol	1.46 mM	-0.11 kcal/mol	-5.40 kcal/mol	709.543
Rivastigmine	-5.65 kcal/mol	72.04 uM	-1.48 kcal/mol	-6.82 kcal/mol	443.259
Spathulenol	-5.21 kcal/mol	151.29 uM	-0.01 kcal/mol	-5.51 kcal/mol	622.526

Table 3: Amino acid Residue Interaction of Lead against *Helicobacter pylori* urease – PDB 1E9Y

Compounds	Interactions	Amino acid residue interactions									
Berberine	2	151 SER	371 GLU	374 THR	378 GLN	445 LYS	475 TYR	563 ALA	567 SER	569 PHE	
Ellagic acid	2	151 SER	371 GLU	374 THR	445 LYS	446 PRO	475 TYR	567 SER	568 ILE	569 PHE	
Gallic acid	2	438 SER	441 PHE	445 LYS	447 ASN	459 GLN	475 TYR				
Huperzine A	2	151 SER	370 GLY	371 GLU	374 THR	444 VAL	445 LYS	446 PRO	475 TYR	567 SER	
Nicotinic acid	2	441 PHE	445 LYS	447 ASN	459 GLN	475 TYR					
Palmatine	2	147 THR	150 ALA	151 SER	374 THR	445 LYS	474 TYR	475 TYR			
Pyridoxine	3	441 PHE	445 LYS	447 ASN	459 GLN	473 VAL	475 TYR				
Rivastigmine	2	150 ALA	151 SER	371 GLU	444 VAL	445 LYS	446 PRO	475 TYR	567 SER	569 PHE	
Spathulenol	2	147 THR	150 ALA	151 SER	371 GLU	374 THR	444 VAL	445 LYS	446 PRO	475 TYR	567 SER

4. Discussion

Natural products, such as traditional preparations and plant extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for the discovery of new drugs due to the unmatched availability of chemical diversity. Natural products include: traditional preparations, plant extracts, either as pure compounds or as standardized extracts [18]. More than 80 percent of the world's population relies on traditional medicine for their primary healthcare, as stated by the World Health Organization (WHO). The long history of human connection with the natural world in Asia is reflected in the region's traditional reliance on herbal remedies [19].

The molecular docking method can be used to model the interaction between a small molecule and a protein at the atomic level. This gives us the ability to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes [20]. The docking process can be broken down into two primary steps: the prediction of the ligand structure as well as its position and orientation within these sites (often referred to as pose), and the measurement of the binding affinity. These two phases are connected to, respectively, sampling methods and scoring schemes, both of which will be addressed in the section devoted to the theory.

Secondary metabolites of plants, also known as phytochemical constituents, include alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds. These phytochemical constituents serve as a defense mechanism for plants against invasion by a wide variety of microorganisms, insects, and other herbivores [21].Flavonoids are a type of hydroxylated phenolic compound that is known to be produced by plants as a reaction to the illness caused by microorganisms [22]. Because it has the ability to trigger leakage of proteins and specific enzymes from the cell, saponin possesses an antimicrobial activity [23]. Tannins attach to proteins that are rich in the amino acid proline and disrupt the process of protein synthesis [24].Within the context of Indian traditional medicine, both the pharmacological activities and therapeutic potential of these phytocomponents are common knowledge. It is well known that medicinal herbs include a wide variety of active principles that have therapeutic significance and has biological action against a number of different ailments [25].

It is critical to take into account the flexibility of the target binding site; nevertheless, this feature is commonly neglected. During the process of chemical recognition, enzymes and receptors are both capable of going through conformational modifications [26]. In certain instances, these structural rearrangements are rather minor, and the ligand is able to fit into a binding site with only a tiny bit of wiggle room. If this is not the case, certain proteins will go through considerable conformational changes, which may involve aspects of their secondary and tertiary structures. Using strategies such as MD [27], one is able to address such flexibility concerns and find solutions.

Docking score of the present investigation implicates the binding affinity between the lead and target higher the negativity in the value that showcase the level of potency of the drug. Development and advancement in the field of computational analysis increased the precision level in identifying the potential drug molecule and deriving its mechanism of action at target site. Selective alterations in the functional groups greatly minimize the non-specific binding and impedes the adverse event at clinical level. Total of 9 bioactive lead compounds were subjected to the docking screening. Out of nine compounds' the lead pyridoxine has maximum of 3 interactions accounts for 100% docking efficiency with the core active amino acid residues present on the target. Followed by this the compounds such as Berberine, Ellagic acid, Gallic acid, Huperzine A, Nicotinic acid, Palmatine, Rivastigmine and Spathulenol ranked second and with the maximum of 2 interactions with the active site of the target *Helicobacter pylori* urease.

5. Conclusion

H. pylori infection has been known to play central role in the development of chronic gastritis, gastric ulcers, duodenal ulcers and gastric cancer. *H. pylori*-derived biomolecules, such as urease have been shown to trigger immune response and contribute to inflammation. Phytotherapeutics either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. In the present investigation the compounds such as Pyridoxine, Berberine, Ellagic acid, Gallic acid, Huperzine A, Nicotinic acid, Palmatine, Rivastigmine and Spathulenol reveals significant binding affinity with that of the target amino acid residues present over the target enzyme *Helicobacter pylori* urease.

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