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Inhibitory Effect of Herbal Compounds on the Oxygen-Insensitive NADPH Nitro Reductase Enzyme of Metronidazole-Resistant *Helicobacter pylori*

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Abstract: *Helicobacter pylori* is a significant risk factor for chronic gastritis, gastric ulcers, and gastric cancer. The purpose of this article is to investigate the potential impact of fifty herbal compounds derived from Ginger and Parsley plants, known for their antibacterial properties on the Oxygen-insensitive NADPH nitro reductase enzyme of metronidazole-resistant *H. pylori*. In the present study, the information on the structure of compounds, the *H. pylori* resistant to metronidazole enzyme, myristicin, and shogaol derivatives were obtained from databases such as ZINC15, RCSB (Protein Data Bank), and PubChem, respectively. Finally, molecular docking was performed with iGemdock2.1 and Molegro Virtual Docker. After molecular docking, four out of the fifty phytocompounds showed the lowest energy and the highest number of interactions with the amino acids at the binding sites. Among these four phytocompounds, the best phytocompound was N-Vanillyloctanamide derived from Ginger. Our molecular docking study suggests that ginger can be introduced as a potential candidate to inhibit the growth of *H. pylori*.

Introduction

Helicobacter pylori is a significant risk factor for chronic gastritis, gastric ulcers, and gastric cancer (1). It is a gram-negative, spiral-shaped, flagellated bacterium that has infected almost half of the world's population (2). The annual rate of infection in developing countries ranges from 4% to 15%, while in developed countries, it is only 0.5% (3). *H. pylori* has developed an acid adaptation mechanism that increases the regulation of periplasmic pH in the harsh acidic environment of the stomach by regulating urease activity (4). Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi, and parasites evolve over time and no longer respond to medicines, making infections harder to treat and increasing the risk of disease spread, severe illness, and death (5). Treatment of *H. pylori* infection comprises a combination of antibiotics and acid-reducing proton pump inhibitors (PPIs). The most common antibiotics are metronidazole, amoxicillin, and clarithromycin. The common PPIs include pantoprazole, emiprazole, lansoprazole and rabiprazole. Combined treatments are usually prescribed for 14 days (6). In most regions of the World Health Organization (WHO), the prevalence of H. pylori AMR to metronidazole is more than 15% (7).

Acquisition of resistance is associated with the mutational inactivation of the rdxa gene, which encodes an oxygen-sensitive NADPH nitroreductase. In *H. pylori*, a mutation in the rdxa gene is significantly associated with metronidazole resistance (8, 9). Studies conducted in Iran have shown that the resistance of *H. pylori* to the metronidazole antibiotic is very high, at about 57.4%. This resistance is about 46.6% in Asian countries. The highest level of resistance of this microorganism to metronidazole is in African countries, where it reaches 97.55% (3).

Essential oils are a mixture of volatile compounds that are produced as secondary metabolites in medicinal plants. According to the International

Organization for Standardization, essential oils are products extracted from plant sources or fruits using steam or water distillation methods. Their chemical compositions are very different based on factors such as the plant, environment, and extraction method. Due to their antimicrobial, antioxidant, anti-inflammatory, and anti-cancer properties, essential oils can be a suitable alternative in the food and pharmaceutical industries (10).

The *in silico* method involves the use of databases, molecular modelling methods, data analysis and mining tools, homology modelling, and pharmacophore modelling (11). An *in silico* study has shown that new 1,2,3 Oxadiazole derivatives have anti-*H. pylori* activities (12). *In silico* analysis has also revealed that *Scrophularia striata* linalool can eliminate *H. pylori* (13), and *in silico* studies have shown that mango ginger is effective against H. pylori (14). In this article, we aim to investigate the potential impact of fifty herbal compounds derived from ginger and parsley plants, known for their antibacterial properties, on the oxygen-insensitive NADPH nitro reductase enzyme of *H. pylori* that is resistant to metronidazole.

Material and Methods

This research was conducted in a descriptive-analytical manner to study the role of different structural parameters in a set of compounds with antibacterial properties. To investigate the relationship between compounds derived from parsley and ginger plants and the resistance of the oxygen-insensitive NADPH nitro reductase enzyme of *H. pylori* to metronidazole, we used the Molegro Virtual Docker model 6 software and iGemdock v2.1.

Molecular Docking by Molegro Virtual Docker Software

Molegro Virtual Docker (MVD) is an integrated platform for predicting protein-ligand interactions. The software handles all aspects of the docking process, from preparing molecules to determining the potential binding sites of the target protein and predicting the binding modes of the ligands. To use the software, we selected the "import molecules" option from the file section and loaded the 3qdl protein file in pdb format. During loading, we removed water and protein ligands by unchecking the boxes next to them. MVD software is a valuable tool for discovering and identifying new drugs based on accurate optimization methods. The data obtained from MVD software have been more accurate in comparison to other software (15). To perform the docking analysis, we prepared the three-dimensional structure of the desired plant compounds and the receptor using various databases, including PDB, ADMETlab, and ZINC15.

Ligand Preparation

To prepare the ligand, we used ChemBio3D and followed these steps: Firstly, we imported the ligand structure file in a compatible format, such as PDB, MOL, or SDF, into ChemBio3D. Then, we removed any unwanted atoms or molecules from the ligand, such as solvent molecules or counterions, using the "Clean Structure" tool. We added hydrogen atoms to the ligand and assigned appropriate protonation states to ionizable groups based on the pH of the system using the "Protonate" tool. Next, we optimized the ligand geometry and minimized any steric clashes between atoms using the "Minimize Energy" tool. Finally, we saved the ligand structure in a compatible format for further analysis or use in molecular docking studies using the "Save As" tool. Overall, ChemBio3D provided a user-friendly interface for ligand preparation and helped us save time and effort in preparing ligand structures for various applications in chemical and biological research.

Receptor Preparation

Chimera is a popular software tool for molecular modelling and visualization that can also be used for receptor preparation. Here are the general steps to prepare a receptor using Chimera: Firstly, obtain the structure of the receptor from a database such as the Protein Data Bank (PDB) or generate it using a homology modelling tool. Then, clean up the receptor structure by fixing errors or missing atoms using Chimera's built-in tools such as "Add H" and "Repair Structure". Any unwanted molecules in the receptor structure can be removed by selecting them using Chimera's "Select" tool and then deleting the selected molecules. Missing loops in the receptor structure can be added using Chimera's "Model Loop" tool. The receptor structure can be optimized using Chimera's "Minimize Structure" tool, which performs energy minimization to optimize the geometry of the structure. Partial charges can be assigned to the receptor atoms using Chimera's "Add Charge" tool, which is essential for molecular docking and other simulations. Once these steps have been completed, the prepared receptor structure can be saved in a format suitable for further simulations, such as PDB or mol2 format. Overall, Chimera is a powerful tool for receptor preparation and can be used to prepare high-quality receptor models for molecular docking, virtual screening, and other simulations.

In this study, we prepared the three-dimensional structure of the protein 3qdl using the PDB database. To accurately examine the information obtained from the MVD software, access to the exact position of the amino acids involved in the ligand-receptor interaction is necessary, and extensive studies were carried out to access this data using the ligand map section of the MVD software. At the time of preparation, the A chain

of 3qdl protein is selected for further steps and docking.

iGemdock software

iGemdock v2.1 is a standalone integrated virtual screening and molecular docking software developed by Jinn-Moon Yang of National Chiao Tung University, Taiwan. This docking software determines the orientation and conformation of the ligand concerning the active site of the protein of interest. Using this software, which is a graphical-automatic system for drug discovery, the integration of docking, screening, post-analysis, and visualization of different ligands can be accomplished. After docking, iGemdock generates protein-ligand interaction profiles of electrostatic (E), hydrogen bonding (H), and van der Waals (V) interactions. In the post-screening analysis, iGemdock infers the pharmacological interactions and clusters the screening compounds based on their interaction profiles and structures. The docked poses were visualized by RasMol. The empirical scoring function of iGemdock was estimated as Energy = vdW+Hbond+Elec. In the present study, we investigated the molecular interaction between the plant compounds, such as parsley and ginger, and the oxygen-insensitive NADPH nitro reductase using iGemdock version 2.1, a specific molecular docking software. This software allows for three-dimensional observation of the interaction of Myristicin and 6-shogaol, as well as similar compounds, with the oxygen-insensitive NADPH nitro reductase, a series of amino acids participating in the interaction, and active functional groups on plant molecules. In our study, to minimize errors, all docking conditions for herbal compounds and standard drugs, including the software used, the number of interactions, the interaction study area, the oxygen-insensitive NADPH nitro reductase enzyme under study, and the docking speed, were considered to be the same. We performed molecular docking between the plant compounds and the oxygen-insensitive NADPH nitro reductase inhibitors using the standard docking method (in which the number of interactions was 70 and the interaction zone diameter was 200 angstroms) with the ability to investigate hydrogen-electrostatic and van der Waals interactions in the entire active site of the enzyme, followed by comparing the results.

Method Validation

Method validation is an essential step in the development and evaluation of computational tools such as iGemdock and MVD. The validation process aims to assess the accuracy, precision, and reliability of the methods used in these tools to ensure that they produce valid and reproducible results.Several studies have been conducted to validate the performance of iGemdock and MVD in predicting protein-ligand interactions. The screening accuracy was generally improved when iGEMDOCK considered the pharmacological interactions. Similarly, another study evaluated MVD's ability to predict binding affinities using a dataset of 109 protein-ligand complexes. The results showed that MVD accurately predicted binding affinities and had a good correlation with experimental data (15, 16). In addition to these studies, the method validation process typically involves several steps, including defining the scope and objectives of the validation study, selecting an appropriate dataset of protein-ligand complexes with known experimental binding affinities, evaluating the accuracy and precision of the tool's predictions using metrics such as root-mean-square deviation (RMSD) and correlation coefficients, assessing the reliability and reproducibility of the tool's predictions, and documenting the validation results in a report or publication. Overall, these studies demonstrate the validity and reliability of iGemdock and MVD in predicting protein-ligand interactions and support their usefulness in drug discovery and development.

To validate the results of MVD and iGemdock simulations, the RMSD was calculated as a measure of the difference between the predicted and reference structures. The RMSD value was calculated separately for both the protein and the ligand. A low RMSD value indicates that the predicted complex structure was in good agreement with the reference structure, suggestive of favorable interactions between the ligand and protein and a predicted binding mode similar to the native binding mode.

Analysis of Docking Results

Biovia Discovery Studio is a commercial-grade graphical visualization tool for viewing, segmenting, analyzing, and modelling data. Firstly, we opened this software and loaded the 3qdl protein PDB file into it. We determined the amino acids of the 3qdl protein binding sites and compared the amino acids of the ligand binding sites with the protein. After performing successive docking to investigate the binding tendency of plant compounds to the receptor, the obtained results were presented in the table. To compare two molecules, Flavin mononucleotide (FMN) and Glycerol (GOL) were studied as control cases.

Binding Site Search

We used this software to identify the amino acids in the binding sites of the 3qdl protein. The amino acids in the binding sites of this protein in all four chains (A, B, C, D) were as follows: Arg16, His17, Ser18, Lys20, Glu34, Pro44, Ser45, Ser46, Asn48, Asn73, Ile142, Cys159, Ile160, Ile161, Gly162, Gly163, Lys198, and Arg200. The results of docking were examined in two dimensions. The first dimension was the energy of each ligand, and the second dimension was the amino acids in the binding sites of each ligand.

Toxicity Studies

ADMET studies are a crucial aspect of drug discovery and development, as they provide valuable information on the pharmacokinetic and pharmacodynamic properties of a drug candidate. The acronym ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity. These five factors are critical in determining the efficacy and safety of a drug. Overall, ADMET studies are essential in optimizing drug development and ensuring that safe and effective drugs are brought to the market. They can be conducted using various in vitro and in vivo methods, such as computational modelling, cell-based assays, animal studies, and clinical trials.

Two databases, ADMETlab 2.0 and ProTox-II, have been used to study toxicity. ADMETlab 2.0 (https://admetmesh.scbdd.com/) is an enhanced version of the widely used ADMETlab for systematical evaluation of ADMET properties, as well as some physicochemical properties and medicinal chemistry friendliness. ProTox-II (https://tox-new.charite.de/protox_II/) is a virtual lab for the prediction of toxicities of small molecules.

Results

Molecular Docking

After performing successive dockings to investigate the binding tendency of plant compounds to the receptor, the obtained results were depicted in a tabular form. Two molecules Flavin Mononucleotide (FMN) and Glycerol (GOL) were studied as control cases. The energy levels were equal to -161.109 and -52.7023 and the amino acids involved were Ser46(C), Tyr47(C), Glu133(A), Arg131(A), Ser88(C), Gln130(A), Leu132(A), and Tyr141(D). The available data showed that N-Vanillyloctanamide, a derivative of the ginger plant, had the lowest (most negative) energy level and the highest number of common amino acids with the main protein in the active site. Its energy level was -124.782.The available data showed that the compound, N-Vanillyloctanamide with energy levels of -124.782, a derivative of the ginger plant has the lowest (most negative) amount of energy with the highest number of common amino acids with the main protein in the active site. **Table 1** shows the results of molecular docking with MVD software. In this table, the results of molecular docking between 3qdl protein and 32 similar compounds related to myristicin, and 18 similar compounds related to shogaol are given. In this table, the zinc code of each compound, the energy obtained from docking (mol dock score), and the number of amino acids in the binding site are given. **Table 2** shows the results of molecular docking with Igemdock software. In this table, the results of molecular docking between 3qdl protein and 32 similar compounds related to myristicin, and 18 similar compounds related to shogaol are given. In this table, the zinc code of each compound, the total energy obtained from docking, the amount of van der Waals energy, H-BOND, and the number of amino acids in the binding site are given.

Table 1. Docking results with Molegro Virtual Docker.

Substance	ZINC ID	Mol dock score (kcal/mol)	Amino acids in binding sites
M1	393470	-102.789	Arg131(A), Gln130(A), Glu133(A), Tyr141(D)
M2	403089	-100.121	Tyr141(D), Gln130(A), Arg131(A)
M3	2146907	-92.6964	Leu132(C), glu133(C), ser46(A), Gln139(A)
M4	2529998	-101.022	Arg131(A), Gln130(A), Tyr141(D), Tyr47(C)
M5	2566085	-89.1359	Tyr71(D), Lys181(A), Ile182(A)
M6	2572638	-99.8687	Arg131(A), Leu132(A)
M7	8727726	-93.224	Ser46(C), Tyr141(D), Gln130(A)
M8	13495667	-93.8757	Leu132(A), Arg131(A)
M9	14489946	-104.402	Arg132(A), Leu132(A), Gln139(C)
M10	14489952	-98.2083	Arg131(A), Gln130(A), Tyr141(D), Tyr47(C)
M11	14680083	-96.8485	Gln130(A), Tyr141(D), Arg131(A)
M12	14818163	-102.409	Arg131(A), Leu132(A), Glu138(C)
M13	14818165	-100.36	Tyr141(D), Gln130(A), Arg131(A), Glu133(A), Leu132(A)
M14	22012904	-102.445	Leu132(A), Ser46(C), Met129(A)
M15	22012908	-101.446	Arg131(A), Tyr141(D)
M16	34182793	-97.4565	Arg131(A), Gln130(A), Tyr141(D)
M17	34186837	-95.9029	Arg131(A), Gln130(A), Tyr141(D)
M18	34186838	-98.1223	Leu132(C), Gln139(A)

M19	38583412	-95.3358	Arg131(A), Leu132(A), Ser46(C)
M20	60249608	-97.2704	Tyr141(D), Gln130(A), Arg131(A)
M21	65336712	-102.6	Leu132(C), Gln130(A), Arg131(C)
M22	65336735	-97.4431	Tyr141(D), Gln130(A), Arg131(A)
M23	95643541	-102.58	Tyr141(D), Gln130(A), Arg131(A)
M24	95934481	-99.9477	Leu132(C), Ser46(C), Phe146(B), Gly162(B)
M25	108374024	-100.203	Tyr141(D), Ala183(D), Ser46(C)
M26	136922029	-97.3492	Arg131(A), Glu138(c), Leu132(A), Ser46(C)
M27	222557475	-103.14	Arg131(A), Gln130(A), Tyr141(D), Tyr47(C)
M28	229338797	-89.1943	Arg131(A), Tyr141(D), Tyr47(C)
M29	229338955	-97.6973	Glu133(C), Gln133(C), Ser46(C), Arg131(C), Gln139(A), Glu138(A)
M30	254568094	-101.274	Leu132(A), Arg131(A), Gln139(C)
M31	257957021	-106.576	Arg131(A), Gln130(A), Tyr141(D), Glu133(A)
M32	1690107322	-92.96.85	Tyr141(D), Gln130(A), Arg131(A)
Sh1	1531857	-121.701	Ser88(D), Gln130(A)
Sh2	1531865	-127.035	Gln138(A), Leu132(C), Arg131(C)
Sh3	1627290	-118.008	Ser88(D), Tyr141(D), Arg131(A)
Sh4	13887692	-124.437	Tyr47(C), Ser46(C), Ser45(C)
Sh5	14708427	-125.739	Tyr141(D)
Sh6	14708433	-120.337	Tyr47(A), Leu132(C)
Sh7	43284710	-124.782	Leu132(C), Arg131(C)
Sh8	58548705	-122.083	Tyr47(C), Glu138(C) , Leu132(A) , Arg131(A)
Sh9	76289028	-122.392	Ser88(D), Lys181(D)
Sh10	95099320	-134.189	Ser88(D), Gln130(A)
Sh11	100294788	-128.878	Ile160(D), Ser46(C)
Sh12	199628214	-118.997	Gln130(A), Leu132(A), Tyr47(C)
Sh13	238744085	-118.24	Glu133(C), Gln139(A)
Sh14	238751661	-123.328	Ile182(D), Leu132(A)
Sh15	238760798	-137.115	Tyr47(A)
Sh16	238777994	-122.739	Gly162(D), Leu132(A)
Sh17	238789702	-114.974	Ser46(C)
Sh18	238789703	-131.987	Ile182(D), Leu132(A)

Table 2. Docking results with IGEMDOCK.

Substance	ZINC ID	Total energy (kcal/mol)	VAN DER WAALS energy (VDW)	H-BOND	Amino acids in binding sites
M1	393470	-77.5122	-63.5135	-13.9987	Ile160, Ser45, Ser46, Ile161, Gly162
M2	403089	-76.77	-66.1	-10.67	Ser45, Ser46, Gln139, Ile160, Pro44
M3	2146907	-74.71	-45.46	-29.25	Arg16, Ser18, Lys198, Arg200, Ser45, Asn48
M4	2529998	-83.32	-59.24	-24.08	Ser18, Ile160, Gly162, Tyr47, Ile161, Ser45, Ser46
M5	2566085	-74.17	-66.2	-7.97	Ser46, Ile160, Ile161, Gly162, Ser45, Gln139
M6	2572638	-73.83	-79.46	-3.37	Ser45, Ser46, Ile160, Ile161, Gln139

M7	8727726	-77.91	-45.43	-32.48	Arg16, Ser18, Lys198, Arg200, Ser45, Asn48
M8	13495667	-83.66	-57.33	-26.33	Ser196, Gln197, Lys198, Arg200, Ser18, Asn48
M9	14489946	-80.37	-66.92	-13.45	Gly162, Ser45, Ser46, Gln139, Ile160, Ile161
M10	14489952	-73	-58.58	-14.42	Ser18, Ile160, Ile161, Gly162, Ser45, Ser46, Tyr47
M11	14680083	-76.32	-65.72	-10.6	Tyr141, Gly162, Ser45, Ser46, Ile160, Ile161, Gln139
M12	14818163	-86.22	-68.69	-17.52	Gly162, Ser45, Ser46, Gln139, Ile160, Ile161
M13	14818165	-83.37	-68.55	-14.82	Gly162, Ser45, Ser46, Ile160, Ile161, Tyr47
M14	22012904	-82.48	-45.17	-35.31	Arg16, Ser18, Lys198, Arg200, His17
M15	22012908	-75.43	-64.05	-10.49	Gly162, Gly163, Ile160, Ile161, Ser45, Ser46, Tyr47
M16	34182793	-70.98	-59.4	-11.58	Arg16, Ser18, Ser196, Arg200, His17, Lys198
M17	34186837	-72.02	-49.25	-22.77	Arg16, Ser18, Ser196, Lys198, Arg200, His17, Gln197, Ser199, Asn48
M18	34186838	-80.71	-61.4	-19.31	Ser18, Cys159, Ile160, Ile161, Ser45, Ser46
M19	38583412	-69.81	-60.65	-9.16	Ser45, Ser46, Ile160
M20	60249608	-74.78	-65.85	-8.93	Ser46, Gln139, Ile160, Ile161, Ser45
M21	65336712	-79.8	-53.49	-26.31	Gly162, Ser46, Ile160, Ile161, Ser45
M22	65336735	-77.29	-52.13	-25.16	Arg16, Ser18, Ser199, Arg200, Gln197, Lys198
M23	95643541	-83.64	-69.67	-13.97	Ser18, Ile160, Ile161, Gly162, Ser45, Ser46
M24	95934481	-85.83	-61.94	-23.89	Ser18, Cys159, Ile160, Ile161, Ser45, Ser46, Gln139
M25	108374024	-77.18	-67.79	-9.39	Ser45, Ser46, Ile160, Ile161, Gln139
M26	136922029	-75.14	-48.2	-26.94	Arg16, Ser18, Arg200, Asn48, Ser45, Tyr47
M27	222557475	-79.65	-64.38	-15.27	Gly162, Ser45, Ser46, Tyr47, Gln139
M28	229338797	-73.18	-51.7	-21.47	Arg16, Ser18, Lys198, Arg200, Asn48
M29	229338955	-88.73	-60.98	-27.75	Gly162, Gly163, Ser45, Ser46, Tyr47, Gln139
M30	254568094	-82.54	-61.41	-21.13	Tyr141, Gly162, Ala183, Ser46, Ser134, Ile160, Ile161
M31	257957021	-82.62	-65.22	-17.4	Gly162, Gly163, Ser45, Ser46, Ile160, Ile161, Gln139
M32	1690107322	-77.04	-49.7	-27.35	Arg16, Ser18, Lys198, Arg200, His17, Gln197
Sh1	1531857	-97.05	-72.19	-24.48	Arg16, Arg200, Tyr47, Asn48, Ser18, Ile160, Ser45, Ser46
Sh2	1531865	-97.18	-83.6	-13.58	Asn73, Gly163, Ser46, Ile160, Ile161, Gly162, Tyr47
Sh3	1627290	-104.03	-81.87	-22.16	Ser45, Ser46, Gly162, Tyr47, Asn48, Gln139, Ser18, Ile160, Ile161, Arg200
Sh4	13887692	-99.98	-80.63	-19.35	Arg16, Lys198, Arg200, Ser18, Ser45, Ser46, Tyr47, Asn48
Sh5	14708427	-94.15	-80.33	-13.82	Gly162, Gly163, Asn48, Ser18, Ile160, Ile161, Ser45, Ser46, Tyr47

Sh6	14708433	-98.24	-86.89	-11.36	Ser45, Ser46, Gln139, Ile160, Ile161, Pro44, Tyr47
Sh7	43284710	-98.01	-80.01	-18	Asn73, Gly162, Gly163, Ser45, Ser46, Ile160, Ile161, Arg200, Pro44
Sh8	58548705	-100.08	-85.14	-14.94	Ser18, Ser46, Gln139, Ile160, Pro44, Ser45
Sh9	76289028	-90.03	-66.95	-23.09	Arg16, Ser18, Ile160, Lys198, Arg200, Pro44, Ser46, Tyr47
Sh10	95099320	-96.67	-83.36	-13.31	Arg200, Ser46, Tyr47, Ser18, Ile160, Lys198, Pro44, Ser45, Asn48
Sh11	100294788	-96.06	-86.02	-10.04	Ser18, Gly162, Tyr47, Ile160, Pro44, Ser45, Ser46, Asn48
Sh12	199628214	-96.22	-84.64	-11.88	Asn73, Gly162, Gly163, Tyr141, Ile160, Ile161, Ser46, Tyr47, Gln139
Sh13	238744085	-91.17	-88.42	-2.74	Tyr47, Ile160, Ile161, Gly162, Ser45, Ser46, Gln139
Sh14	238751661	-96.89	-89.89	-7	Gly162, Tyr47, Ile160, Lys198, Arg200, Ser45, Ser46
Sh15	238760798	-96.01	-76.2	-19.81	Arg16, Ser18, Lys20, Lys198, Arg200, Ser45, Ser46, Tyr47
Sh16	238777994	-92.37	-84.28	-8.09	Ser18, Gly162, Ile160, Ile161, Ser45, Ser46, Gln139
Sh17	238789702	-92.73	-79.59	-13.14	Ser46, Ser134, Gln139, Ile160, Ile161, Gly162, Ser45
Sh18	238789703	-99.74	-79.11	-20.64	Arg16, Ser18, Lys20, Lys198, Arg200, Ile160, Ser45, Ser46

The BIOVIA Discovery Studio software was used to find amino acids in binding sites of 3qdl protein. The amino acids in binding sites of this protein in all four chains A, B, C, and D were as follows: Arg16, His17, Ser18, Lys20, Glu34, Pro44, Ser45, Ser46, Asn48, Asn73, Ile142, Cys159, Ile160, Ile161, Gly162, Gly163, Lys198, and Arg200. The results of docking were examined in two dimensions. The first dimension is in terms of the energy of each ligand and the second dimension is in terms of the amino acids in the binding sites of each ligand.Among the results of molecular docking with MVD software, out of 50 molecules, Myristicin, with a zinc code of 25795702 and a MolDock score of -106.567, and Shogaol, with a zinc code of 238760798 and a total energy of -137.115, had the lowest energy levels (the most negative). Similarly, among the results of molecular docking with iGemdock software, Myristicin, with a zinc code of 229338955 and a total energy of -88.73, and Shogaol, with a zinc code of 1627290 and a total energy of -104.03, had the lowest energy levels (the most negative).

According to the results of BIOVIA Discovery Studio software and molecular docking with MVD, Ligand software with zinc code equal to 95934481 related to Myristicin with two common amino acids in binding sites with the main protein which were Ser46(c) and Gly162(b) and the ligand with zinc code equal to 100294788 related to Shogaol substance with two common amino acids in binding sites with the main protein which were Ser46(c) and Ile160(d) were selected. According to the results of BIOVIA Discovery Studio software and molecular docking with Igemdock software, the ligand with zinc code equal to 257957021 related to Myristicin substance with six amino acids in binding sites in common with the main protein, which were Ser45(c) Gly163, ser46, Ile160, Ile161, and Gly162 and the ligand with zinc code equal to 43284710 related to Shogaol substance with nine common amino acids in binding sites with the main protein which were Ser45, Ser46, Ile160, Ile161, Gly163, Asn73, Arg200, Pro44, and Gly162 were selected. In cases where the number of common amino acids in binding sites of two or more zinc codes was equal, the code with the lowest energy (the most negative) was selected.

The result of docking with MVD software was that the mol dock score was equal to -85.1506 and the number of common amino acids in binding sites with the output of Biovia software was equal to one active site, which was Ser46(c). The result of docking with iGemdock software was that the total energy was equal to -87.9 and the number of common amino acids in binding sites with the output of Biovia software was equal to four active sites, which were: Arg16, Lys198, Ser18, Arg200. Among the tested phytocompounds, no phytochemical was able to outperform the control in terms of binding energy. Therefore, considering the importance of the active site residues, this dimension was prioritized.

Figure 1. 3D structure of 3qdl and its ligands.

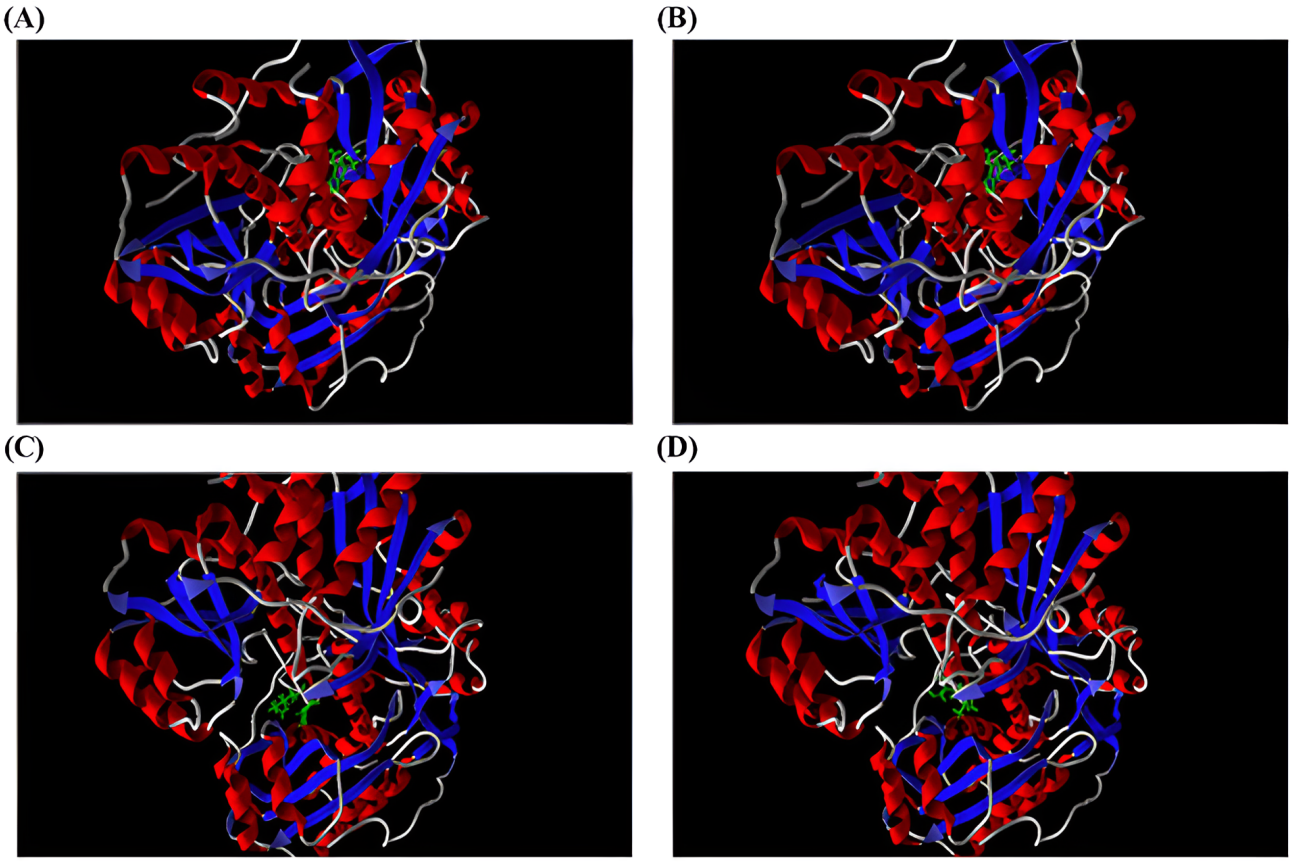

Figure 2. Docking result for 4 best molecules with MVD (A) ZINC ID: 95934481, (B) ZINC ID: 100294788, (C) ZINC ID: 257957021, and (D) ZINC ID: 43284710.

Method Validation

The RMSD values for the predicted and reference structures were calculated using the superimposition of the native ligand and receptor. The RMSD values for each protein were compared with a main protein value of 2.543. The results were as follows:

- Protein a: RMSD = 3.00
- Protein b: RMSD = 1.00
- Protein c: RMSD = 0.10
- Protein d: RMSD = 3.4

Based on these values, it was observed that protein C had the lowest RMSD value, indicating that it was the most similar to the main protein. Protein A and protein B had relatively low RMSD values, indicating that they were also somewhat similar to the main protein. Protein d had the highest RMSD value, indicating that it was the most different from the main protein. Overall, the differences between the proteins were relatively small, as the highest RMSD value was only 3.4 (compared to 2.543 for the main protein). However, the interpretation of these values depended on the context of the analysis and the specific goals of the research. Smaller RMSD value indicated that the ligand had bonded more with the active site amino acids.

Table 3. Toxicity studies using the ADMETlab website for four selected molecules.

Zinc ID	hERG Blockers	H-HT	DILI	AMES Toxicity@	Rat Oral Acute Toxicity	FDAMDD*	Skin Sensitization	Carcinogencity	Eye Corrosion	Eye irritation	Respiratory Toxicity
95934481	.031	.806	0.148	0.522	0.454	0.656	0.512	0.932	0.83	0.067	0.93
25757021	0.017	0.321	0.177	0.041	0.019	0.635	0.682	0.887	0.019	0.755	0.084
100294788	0.065	0.422	0.432	0.159	0.587	0.21	0.953	0.598	0.159	0.962	0956
43284710	0.159	0.09	0.054	0.075	0.041	0.029	0.844	0.045	0.006	0.088	0.074

Note: *The maximum recommended daily dose provides an estimate of the toxic dose threshold of chemicals in humans. @The test for mutagenicity. hERG = human ether-a-go-go related gene; DILI = drug-induced liver injury; H-HT = human hepatotoxicity.

Table 4. Toxicity studies using the ProTox-II website for four selected molecules.

Predicted toxicity class	LD50 (mg/kg)	Zinc ID
3	170	95934481
4	1000	257957021
4	1250	43284710
5	2300	100294788

Note: Class I: fatal if swallowed (LD50 ≤ 5); Class II: fatal if swallowed (5 < LD50 ≤ 50); Class III: toxic if swallowed (50 < LD50 ≤ 300); Class IV: harmful if swallowed (300 < LD50 ≤ 2000); Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000); and Class VI: non-toxic (LD50 > 5000).

Toxicity Studies

The *in silico* toxicity studies with the ADMETlab and ProTox-II tools are given in **Table 3** and **Table 4**, respectively. The study indicates that all the phytocompounds have an LD50 higher than 170 mg/kg body weight and might be toxic if ingested above the recommended LD50. According to **Table 3** and **4**, which is related to toxicity studies, the four phytocompounds might show signs of toxicity. However, the in silico results are based on a model that is trained from a group of compounds with known toxicity, and hence, the *in silico* results might not be completely accurate due to differences in the chemical structures of the training sets and the test sets.

Discussion

N-Vanillyloctanamide compound, which was derived from the ginger plant and Shogaol substance, had inhibitory effects against the 3qdl protein of *H. pylori* bacteria, which indicates that plant compounds can be introduced as a potential antibiotic. O'Mahony *et al*. (2005), Ebrahimzadeh Attari et al. (2019) and Hedieh Yousef-Nezhad *et al*. (2017) proved that parsley and ginger showed inhibitory activity against *H. pylori* (17-19). Weerasekera *et al*. (2008) confirmed in a study that parsley has bactericidal properties, but the complete inhibition of bacteria was not achieved in 60 minutes (20). Another study assessed the effects of curcumin, a polyphenolic compound found in turmeric, on the Oxygen-insensitive NADPH nitro reductase enzyme of *H. pylori*. The study reported that curcumin inhibited the enzyme's activity, reduced *H. pylori* growth, and increased metronidazole sensitivity in *H. pylori* strains resistant to the antibiotic (21). Ebrahimzadeh Attari *et al*. (2019) concluded that ginger can be considered a useful complementary therapy for functional dyspepsia (22). Azadi *et al*. (2019) showed that a combination of cinnamon and ginger extracts can have inhibitory effects against *H. pylori* (23). Sistani Karampour *et al*. (2019) showed that ginger can have protective effects on gastric ulcers (24). Al Yahya *et al*. (1989) showed that ginger has cytoprotective and anti-ulcerogenic effects (25).

Conclusion

In conclusion, the findings of this study provide promising insights into the development of new treatment strategies for *H. pylori* infections, especially in cases where antibiotic resistance occurs, and suggest that targeting the Oxygen-insensitive NADPH nitro reductase enzyme may be a promising approach for the development of new and more effective drugs for the treatment of *H. pylori* infections. Our study demonstrated that ginger might have an inhibitory effect on the oxygen-insensitive NADPH nitro reductase enzyme of *H. pylori* strains that are resistant to metronidazole. Among the tested phytocompounds, no phytochemical was able to outperform the control in terms of binding energy. Therefore, considering the importance of the active site residues, this factor was prioritized for choosing phytocompounds with potential activity. Further studies are needed to evaluate the efficacy and safety of these compounds and to investigate their potential use in combination with other antibiotics to enhance their antimicrobial activity against *H. pylori*.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

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Not applicable.

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