



# Molecular docking of a set of flavonoid compounds with *Helicobacter pylori* virulence factors CagA and VacA

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## ABSTRACT

**Introduction:** Cytotoxin associated gene A (CagA) and vacuolating cytotoxin A (VacA) proteins are the main *Helicobacter pylori* virulence factors. These toxins are associated with severe gastric diseases. Flavonoids are plant secondary metabolites that have shown great antibacterial effects. This work aimed to study the interaction of a set of flavonoid compounds with CagA and VacA proteins using molecular docking.

**Methods:** A set of 54 flavonoid compounds were used in this study, and 36 of which passed the Lipinski rules of 5. The 3D structures of CagA and VacA proteins were obtained from the Protein Data Bank. The molecular docking was performed using AutoDock Vina software and the results were expressed in terms of binding energies (kcal/mol). Protein-ligand interactions were analyzed using PyMOL software.

**Results:** For the CagA protein, the licochalcone A molecule showed the highest binding affinity (-8 kcal/mol). For the VacA protein, the galangin, luteolin, and apigenin molecules showed the highest binding affinity (-8.9, -8.5, and -8.2 kcal/mol, respectively). Interactions of the licochalcone A, galangin, luteolin, and apigenin with CagA and VacA proteins involved their hydroxyl groups and/or their carbonyl groups.

**Conclusion:** Our study showed that these compounds might have the potential for their development into drugs for controlling *H. pylori* pathogenicity.

### Implication for health policy/practice/research/medical education:

Flavonoids are natural molecules produced and used by plants as a defense against biotic stresses. Some of these molecules showed great affinities with *H. pylori* virulence factors (CagA and VacA). Hence, these compounds can be used as candidates for development of new drugs against *H. pylori*.

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## Introduction

*Helicobacter pylori* is a bacteria that colonizes the stomach of almost half of the world's population (1). Infection with this bacteria is characterized by chronic inflammation of the stomach that can progress to several gastric illnesses such as gastric ulcer, gastric cancer, and MALT lymphoma (2). CagA and VacA proteins are the most studied virulence factors of *H. pylori* and are associated with the severity of gastric diseases. The CagA protein is characterized by a tertiary structure composed of a structured N-terminal region, as well as an intrinsically

disordered C-terminal region (3). The N-terminal region of the CagA protein allows it to interact with several host proteins and trigger different signaling pathways (4). The CagA N-terminal region has been shown to bind to the apoptosis-stimulating of p53 protein 2 (ASPP2), a tumor suppressor protein, causing the inhibition of the p53 protein apoptotic function and activating its degradation (5).

The VacA protein is characterized by the presence of two domains, p33 and p55, involved respectively in the formation of ion channels in the cytoplasmic membrane

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and the binding of the toxin to host cells (6). To date, only the tertiary structure of the p55 domain of the VacA protein has been identified (7).

The emergence of antibiotic-resistant bacteria has become the leading cause of failure in the treatment of infectious diseases (8). The World Health Organization has ranked *H. pylori* among the 12 most resistant bacteria in the world (9). Recently, a promising approach consisting of the inhibition of bacterial virulence factors has emerged (10). Rather than inhibiting the cellular components necessary for the growth or viability of the bacteria, these compounds would decrease the severity of infection by interfering with aspects of bacterial pathogenesis (11).

Since pharmaceutical development relies on natural products to provide biological active components, the screening of natural antibacterial agents has been widely studied and has even become a new engine for the discovery of antibacterial drugs (12-14).

Among these natural molecules, flavonoids are a class of polyphenolic compounds produced by plants as secondary metabolites and act as a defense system against different biotic and abiotic stresses (15). Several studies have shown that flavonoids have antibacterial activity (10,16,17).

Due to the antibacterial effects of flavonoids, a molecular docking study of a set of flavonoid compounds with *H. pylori* virulence factors (CagA and VacA) was performed.

## Materials and Methods

### The tertiary structure of CagA and VacA proteins

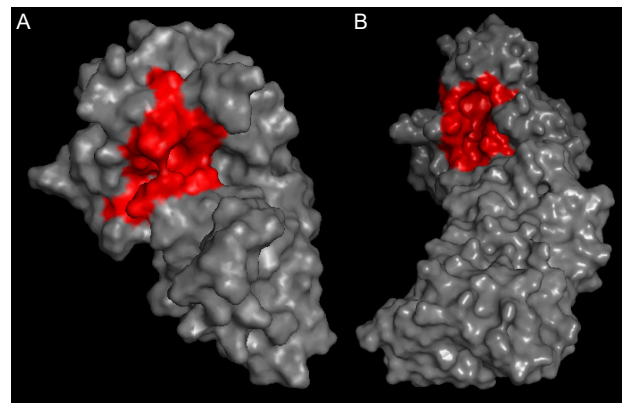
The tertiary structures of CagA and VacA proteins were obtained from the Protein Data Bank (PDB). For the CagA protein, we used the tertiary structure of the CagA N-terminal region (residues 24-824) resolved by Hayashi with the PDB ID 4DVY (3). The portion of the N-terminal region of the CagA protein (residues 24-221) responsible for its interaction with the ASPP2 protein was isolated using the PyMOL software version 2.0.2 (Figure 1A), and the residues involved in this interaction were selected from Nešić et al study (18).

For the VacA protein, we used the tertiary structure of the p55 domain resolved by Gangwer with the PDB ID 2QV3 (7). The CASTp server was used to determine the best binding site of the VacA protein (19) (Figure 1B).

The residues of the CagA and VacA proteins forming the binding sites are listed in Table 1.

### Structural optimization of CagA and VacA proteins

The tertiary structure of CagA and VacA proteins was optimized using the molecular dynamics simulation software Gromacs version 5.1.2, with the AMBER 99SB force field and the TIP3P water model. The energy minimization consisted of a 6000 step simulation using the steepest descent algorithm. The structures obtained had minimal energy confirmation which ensured their stability. These structures were then used for molecular docking.



**Figure 1.** Surface representation of the N-terminal region of the CagA protein (A) and the p55 domain of the VacA protein (B). The binding sites of the CagA and VacA proteins are indicated in red.

**Table 1.** The binding sites residues of CagA and VacA proteins

<i>H. pylori</i> virulence factors	Residues forming the binding site
CagA	Phe26, Ile105, Val107, Thr111, Phe114, Ile175, Trp212, Ile215, Phe219, Phe221
VacA	Thr641, Asp669, Ala671, Thr672, Phe674, Tyr675, Lys676, Pro677, Lys680, Tyr729, Asn733, Arg734, Thr737, Cys738, Val739, Val740, Arg741, Asp745, Ala748, Cys749, Ala752

### Ligands database

A set of 54 flavonoid compounds obtained from the PubChem database was used in this study (Table 2). Before proceeding with the molecular docking, filtering of these 54 flavonoid compounds was carried out based on the Lipinski rules of 5 (20) using the Molinspiration online server. This step makes it possible to keep the compounds capable of fulfilling the criteria of absorption, distribution, metabolism, and excretion, essential for a compound to be considered as a potentially active drug orally in the human body.

### Molecular docking

The AutoDock Vina software version 1.1.2 was used for molecular docking of flavonoid compounds with CagA and VacA proteins. The grid box enclosing the CagA and VacA proteins binding sites were calculated using the AutoDock Vina plug-in available on PyMOL and developed by Daniel Seeliger. The position and dimensions of the box are shown in Table 3. Molecular docking results were expressed in terms of binding energies (kcal/mol), and protein-ligand interactions were analyzed using PyMOL software version 2.0.2.

## Results

### Analysis of bioavailability of the flavonoid compounds

The bioavailability analysis of the 54 flavonoid compounds

**Table 2.** Names, PubChem ID, and the status of the Lipinski rules of 5 of the 54 flavonoid compounds used in this study

Compounds names	PubChem ID	5 Lipinski rules status
Abyssinones	10408069	Fail
Apigenin	5280443	Pass
Baicalcin	5281605	Pass
Baicalin	64982	Fail
Bartericin A	12136209	Fail
Biochanin	5280373	Pass
Catechin	9064	Pass
Chrysin	5281607	Pass
Daidzein	5281708	Pass
Daidzin	107971	Pass
Dihydromyricetin	161557	Fail
Diosmetin	5281612	Pass
Epicatechin	72276	Pass
Epigallocatechin	72277	Fail
Eriodictyol	440735	Pass
Fisetin	5281614	Pass
Flavone	10680	Pass
Flavylum	145858	Pass
Formononetin	5280378	Pass
Galangin	5281616	Pass
Genistein	5280961	Pass
Genistin	5281377	Fail
Hesperidin	10621	Fail
Hesperitin	72281	Pass
Isobavachalcone	5281255	Pass
Isolupalbigenin	26238934	Fail
Isoorientin	114776	Fail
Kaempferol	5280863	Pass
Licochalcone A	5318998	Pass
Luteolin	5280445	Pass
Macluraxanthone	5281646	Fail
3'-O-Methyldiplacol	21607150	Pass
Morin	5281670	Pass
Myricetin	5281672	Fail
Myricitrin	5281673	Fail
Naringenin	932	Pass
Naringin	442428	Fail
Nobiletin	72344	Pass
Panduratin A	6483648	Fail
Peonidin	164544	Pass
Puerarin	5281807	Fail
Quercetin	5280343	Pass
Rutin	5280805	Fail
Sakuranetin	73571	Pass
Scopoletin	5280460	Pass
Scutellarein	5281697	Pass
Tangeritin	68077	Pass
Taxifolin	439533	Pass
3,5,6,7-Tetramethoxyflavone	471721	Pass
Theaflavin	135403798	Fail
Tricin	5281702	Pass
Trihydroxyflavone	17840571	Pass
Warangalone	5379679	Fail
Wogonin	5281703	Pass

**Table 3.** Docking box parameters used in this study

<i>H. pylori</i> virulence factors	Box dimensions in Å <sup>2</sup>			Box coordinates in Å <sup>2</sup>		
	X	Y	Z	X	Y	Z
CagA	68	69	51	51.11	55.11	54.1
VacA	64	64	73	58.73	81.74	47.73

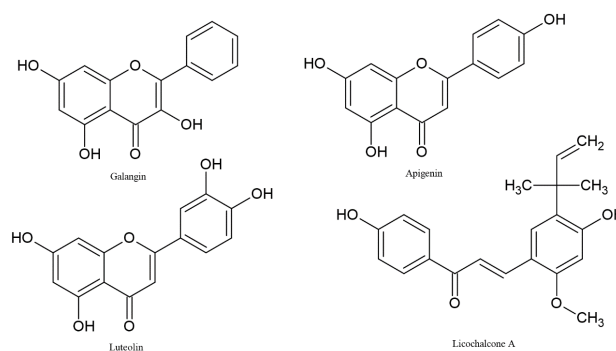
by the Lipinski rules of 5 showed that 36 compounds met all criteria (Table 2). These compounds were then used for molecular docking.

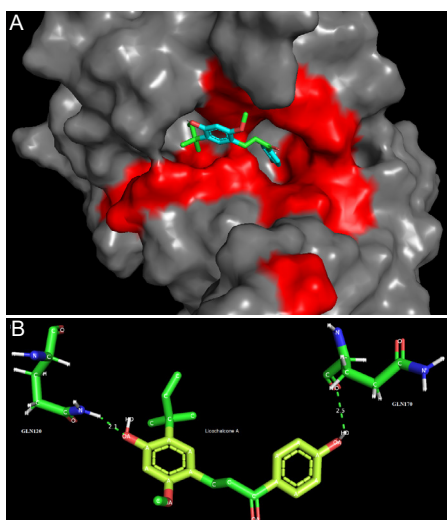
### Analysis of molecular docking results

For the CagA N-terminal region, the licochalcone A molecule (Figure 2) showed the highest binding energy (-8 kcal/mol). The hydroxyl groups at the 4 and 4' positions located respectively on the A and B rings of licochalcone A interacted with the Gln120 hydrogen atom and Gln170 oxygen atom of the CagA N-terminal region with distances of 2.1 and 2.5 Å<sup>2</sup>, respectively (Figure 3). For the VacA protein, the galangin, luteolin, and apigenin molecules (Figure 2) showed the highest binding energies (-8.9, -8.5, and -8.2 kcal/mol, respectively).

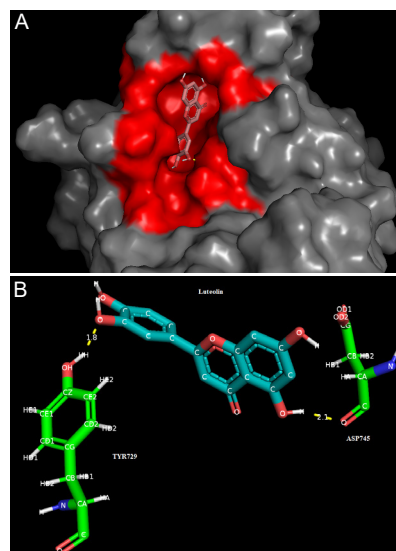
The interaction of the galangin with the VacA protein involved its hydroxyl and carbonyl groups. The hydroxyl group at position 3 of the C ring interacted with the Thr737 oxygen atom with a distance of 2.1 Å<sup>2</sup>. This same hydroxyl group interacted also with the Val739 oxygen and hydrogen atoms with distances of 3.2 and 2.5 Å<sup>2</sup>, respectively. Also, the Val739 hydrogen atom interacted with the galangin carbonyl group located at position 4 of the C ring with a distance of 2.2 Å<sup>2</sup>. Finally, the galangin hydroxyl group at positions 5 and 7 of the A ring interacted respectively with the Asp745 oxygen atoms with distances of 2.3 and 2.6 Å<sup>2</sup> (Figure 4).

The interaction of luteolin with the VacA protein involved its hydroxyl groups. The hydroxyl group located at the 5 position of the A ring interacts with the Tyr729 hydrogen atom with a distance of 1.8 Å<sup>2</sup>, while the hydroxyl group located at the 3' position of the B ring interacted with the Asp745 oxygen atom with a distance of 2.1 Å<sup>2</sup> (Figure 5).

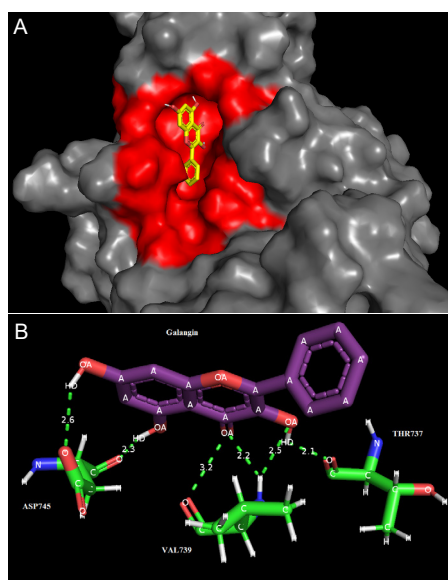
**Figure 2.** The two-dimensional structure of the flavonoid compounds with the high binding energies from molecular docking.



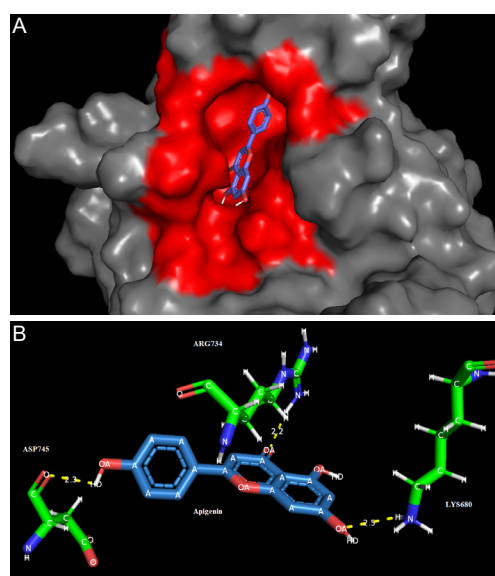
**Figure 3.** Interaction of licochalcone A molecule (sticks) with the CagA N-terminal region (surface mode) (A). Interaction of licochalcone A molecule with the Gln120 and Gln170 amino acids of the CagA N-terminal region (B).



**Figure 5.** Interaction of luteolin molecule (sticks) with the VacA protein (surface mode) (A). Interaction of luteolin with the amino acids Tyr729 and Asp745 of the VacA protein (B).



**Figure 4.** Interaction of the galangin molecule (sticks) with the VacA protein (surface mode) (A). Interaction of the galangin molecule with the amino acids Thr737, Val739, and Asp745 of the VacA protein (B).



**Figure 6.** Interaction of apigenin molecule (sticks) with the VacA protein (surface mode) (A). Interaction of apigenin molecule with the amino acids Lys680, Arg734, and Asp745 of the VacA protein (B).

The interaction of apigenin with the VacA protein involved its carbonyl and hydroxyl groups. The carbonyl group at the 4 position of the C ring interacted with the Arg734 hydrogen atom with a distance of  $2.2 \text{ \AA}$ . The hydroxyl group at the 4' position of the B ring interacted with the Asp745 oxygen atom with a distance of  $2.3 \text{ \AA}$ , and the hydroxyl group at the 7 position of the A ring interacted with the Lys680 hydrogen atom with a distance of  $2.5 \text{ \AA}$  (Figure 6).

## Discussion

The inefficiency of currently available antibiotics encourages the search for new antibacterial agents capable of countering this growing resistance. The efficacy of the treatment for the eradication of *H. pylori* infection has decreased considerably due to the resistance of this bacterium to antibiotics (21,22). *H. pylori* resistance rate to antibiotics (clarithromycin, metronidazole, and levofloxacin) exceeded the rate of 15% in almost 65 countries (9). The antibacterial effects of flavonoids

include inhibition of bacterial attachment to host receptors, inhibition of biofilm formation, and neutralization of bacterial toxins (23,24). Several works have reported a flavonoid inhibitory activity against *H. pylori* (25–28). The antibacterial potential of flavonoids led us to study the interaction of a set of flavonoid compounds with the main *H. pylori* virulence factors (CagA and VacA).

The docking of the 36 flavonoid molecules with the CagA N-terminal region showed that the licochalcone A molecule had the highest binding energy. Licochalcone A, derived from the licorice root (*Glycyrrhiza glabra* or *Glycyrrhiza radix*), is a flavonoid belonging to the chalcones family and used for the treatment of gastric ulcers and inflammations (29,30). The antibacterial effects of chalcones against several bacterial species, including *H. pylori*, have been shown by multiple studies (31–35).

In our study, the interaction of licochalcone A with the CagA N-terminal region involved their hydroxyl groups. The antibacterial activity of flavonoids depends on their structures, precisely the substitutions on their aromatic cycles. Indeed, authors have shown that hydroxyl groups at the 4 and 4' positions of the A and B rings are essential for the antibacterial activity of chalcones (36–38). It was shown that chalcones extracted from the licorice root (licochalcone A and licochalcone E) inhibit bacterial infection by decreasing their gene expression, inhibiting their growth, and reducing their toxin production (39). The licochalcone A showed an inhibitory effect against the growth of *H. pylori*, even on resistant strains (40).

Galangin was the molecule with the highest binding energy with VacA protein. This molecule belongs to the class of flavonols and is present in the small galanga (*Alpinia officinarum*) and propolis. Galangin has excellent anti-cancer, anti-inflammatory, and antioxidant effects (41–43).

Interaction of galangin with the VacA protein involved its hydroxyl and carbonyl groups on the C ring as well as the hydroxyl group on the A ring. It was shown that hydroxyl groups on the A (at 5 and 7 positions), B, and C rings increased the antibacterial activities of flavonols (44,45). However, the hydroxylation of the B ring at position 4' decreases this effect (46). The galangin molecule is characterized by multiple hydroxylation groups at A (at 5 and 7 positions) and C rings (at 3 position). As a result, significant antibacterial effects of this molecule have been reported by several studies (47–50).

The other two compounds (luteolin and apigenin) with good binding energy to the VacA protein belong to the class of flavones. Luteolin is a molecule found in several vegetables (carrots, peppers, celery, olive oil) and medicinal plants (51), and has several biological effects such as antioxidant, anti-cancer, and anti-inflammatory effects (51). The interaction of luteolin with the VacA protein showed binding energy of -8.5 kcal/mol. Numerous studies have reported the antibacterial activity of luteolin

on several bacterial species (52–54). An inhibitory effect of this molecule on the growth of *H. pylori* has been demonstrated (55). In this study, the luteolin molecule interacted with the VacA protein by its hydroxyl groups. Although the antibacterial effects of luteolin have been demonstrated in several studies, the structure-activity mechanism of this molecule is still unknown. The antibacterial effect of a given flavonoid is determined by the number and the positions of its hydroxyl groups on its rings (15). In the case of flavones, possessing at least one hydroxyl group in the A ring (at position 7) is vital for the antibacterial activity, and in other positions (5 and 6) can increase this effect (56). Hence, the presence of hydroxyl groups in positions 5 and 7 of the A ring could confer to luteolin a significant antibacterial effect, which may also explain its high binding energy.

The apigenin molecule is among the most common flavonoids in the plant kingdom (57). It is mainly found in vegetables (parsley, celery, onions), fruits (oranges), herbs (chamomile, thyme, oregano, basil) and herbal drinks (tea, beer, wine) (58). The apigenin was shown to have multiple biological properties including anti-cancer, anti-inflammatory, and antioxidant effects (59–64).

The apigenin molecule interacts with the VacA protein by its hydroxyl and carbonyl groups. The potent antibacterial activity of the apigenin against a number of bacteria has been assessed by several studies (65–67). As discussed above, the antibacterial activity of flavones is directed by the number and positions of the hydroxyl groups they contain. The hydroxylation at position 7 of the A ring is required for the antimicrobial activity of flavones. As a result, the hydroxyl groups of apigenin could be at the origin of its antibacterial activity.

Numerous works have investigated the interaction of natural molecules against *H. pylori* virulence factors. *In vitro* and *in vivo* studies suggest that isoflavone, flavonol, and chalcone compounds inhibit the urease activity, which plays an important role in the colonization of the gastric mucosa by *H. pylori* (26–28,68). Several flavone derivatives and other polyphenols present in vegetables and plants inhibit ionic conduction and urea by *H. pylori*, as well as vacuolization induced by the VacA protein (25). Moreover, Srivastava et al studied the molecular docking of the curcumin molecule with *H. pylori* virulence factors (CagA, VacA, and urease), and showed that this molecule presents good affinities with the urease (69). All these studies converge toward the idea that the approach of neutralizing bacterial virulence factors may be an alternative to the strategy commonly used in the treatment of infectious diseases, which could have an impact on reducing the high antibiotic resistance rate, specifically for *H. pylori* infection.

## Conclusion

Our study showed that from the 36 flavonoids molecules

that passed the Lipinski rules of 5, the licochalcone A molecule showed the highest binding affinity with the CagA protein, while the galangin, luteolin, and apigenin molecules showed the highest binding affinity with the VacA protein. We also demonstrated that interactions of all of these molecules with CagA and VacA proteins involved their hydroxyl and/or their carbonyl groups, which are essentials for their antibacterial activity. Therefore, it seems that these molecules could play an inhibitory role in the signaling and recognition processes induced by CagA and VacA proteins, and thus may be used as potential candidates for designing new antibiotics.

#### Authors' contributions

MRJ, AK, and FM designed the study. MRJ carried out the study and wrote the manuscript. GB, IE, HB, KZ, and HL revised the manuscript. All authors read and approved the final version of the manuscript.

#### Conflict of interests

Authors declare no conflict of interest.

#### Ethical considerations

No ethical approval was required for the conduct of this study. Text plagiarism, misconduct, manipulation or appropriation, data fabrication, falsification, redundant publication as well as duplicate submissions have been carefully observed by authors.

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