# A REVIEW ON CURRENT TRENDS IN *Helicobacter pylori* MANAGEMENT WITH MEDICINAL PLANTS AND ITS CONSTITUENTS

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**ABSTRACT**

*Helicobacter pylori is a bacterium associated with gastric diseases and disorders of the upper gastrointestinal tract. The gram-negative bacterium Helicobacter pylori is known as a persistent colonizer of the human stomach, and this bacteria is also involved in extra-intestinal diseases. In 1994, the International Agency for Research on Cancer, World Health Organization classified H. pylori as a class 1 carcinogen, the only bacterium given this classification. Besides, the emergence of H. pylori resistance to antibiotics has been a major clinical challenge in the field of gastroenterology, and this concern has been shown an increasing tendency in many regions of the world. To overcome the current circulating difficulties, new potential therapeutic targets were uncovered to find active substances for the treatment of H. pylori infection. Several medicinal plants and their isolated compounds have been reported for their antimicrobial activity against H. pylori. It is demonstrated that they are efficacious against H. pylori strains that are resistant to drugs. The mechanism of action of many of these plant extracts and plant-derived compounds is different from that of conventional antibiotics. Therefore, natural compounds are emerging as a potential source of raw materials with diverse mechanisms of action. Some commonly known mechanisms can be listed as anti-urease activity, anti-adhesive activity, anti-inflammatory and gastroprotective activity, and effects on the oxidative stress process. Recently, new classes of drugs with reasonable antibacterial mechanisms against H. pylori have also been mentioned, including (1) anti-biofilm agents, (2) anti-virulence molecules (anti-VacA, anti-CagA agents, toxin BabA and LPS inhibitors, anti-motility agents, Helicobacter pylori quorum sensing inhibitors), (3) mucolytic agents, and (4) compounds that impact on essential proteins in the physiology of H. pylori such as inosin-5'-monophosphate dehydrogenase and HsrA inhibitors. This review article aims to summarize current*

*prospects, identify possible novel targets, and be considered as a complementary therapy in the eradication treatment against Helicobacter pylori.*
***Keywords:*** *anti-biofilm, anti-mucolytic, anti-virulence, Helicobacter pylori, HsrA inhibitors, Inosin-5′-monophosphate dehydrogenase inhibitors.*

## I. INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a gram-negative, microaerophilic, and spiral bacterium that was first cultured and identified by Marshall and Warren. This organism mainly colonizes on the surface of the gastric mucosa and is associated with chronic gastritis, gastric ulcers, gastric mucosa-associated tissue (MALT) lymphoma, gastric cancer, and other disorders of the upper gastrointestinal tract [1]. In addition, resistance of *H. pylori* to antibiotics has reached alarming levels worldwide, and the efficacy of the *H. pylori* eradication treatment has decreased dramatically because of antibiotic resistance. Historically, conventional antibiotics and proton pump inhibitors (PPIs) have been used in triple therapy to treat *H. pylori.* However, due to widespread metronidazole and clarithromycin resistance, *H. pylori* eradication rates have dropped to unacceptable levels. Certain studies claim that typical triple therapy did not even come close to eliminating *H. pylori* [2]. Researchers have explored a variety of strategies to mitigate the impacts of antibiotic resistance, including changing medication regimens, increasing drug dosages, or prolonging treatment periods [3]. According to the study of Li et al (2023), there is currently no *Helicobacter pylori* vaccine for humans on the market [4].

Therefore, finding more new drugs derived from medicinal plants and their compositions with unique mechanisms is always necessary in this day and age. With their many modes of action in both *in vitro*, *in vivo* assay, and in small-scale clinical trials, natural compounds are becoming more and more of a viable source of raw materials in *Helicobacter pylori* treatment. Several widely recognized processes include anti-urease, anti-adhesive, anti-inflammatory, and gastroprotective properties, in addition to their impact on the process of oxidative stress in bacterial cells [5, 6]. To improve the rate of successful eradication of *H. pylori*, various complementary therapies with novel targets have been uncovered such as (1) anti-biofilm agents, (2) anti-virulence molecules (anti-VacA, anti-CagA agents, toxin BabA and LPS inhibitors, anti-motility agents, *H. pylori* quorum sensing inhibitors), (3) mucolytic agents, and (4) inosin-5’-monophosphate dehydrogenase and HsrA inhibitors. Therefore, this review article aims to analyze current trends regarding these promising mechanisms in *Helicobacter pylori* management.

## II. CONTENTS

### 2.1. Natural anti-biofilm agents targeting *H. pylori* infection

#### 2.1.1. Biofilm of *Helicobacter pylori*

One of the factors that affect the effectiveness of conventional eradication therapies is the ability of *H. pylori* to form biofilms. Previously, most studies focused only on the planktonic form (free-living) of *H. pylori*. However, several recent studies indicate that *H. pylori* can develop biofilm structures when observed in both *in vitro* and *in vivo* models. The presence of biofilm has been observed in the gastric mucosa, glands, and gastrointestinal tract of mice or patients infected with *H. pylori*. The first evidence of the presence of *H. pylori* biofilm during infection of the human gastric mucosa was reported in articles by Carron and Coticchia in 2006. The studies using biopsy samples and SEM

analysis have demonstrated the dense presence of bacterial biofilm on the gastric mucosal surface of patients positive for *H. pylori* with a rate of more than 97.3% [7], [8].

After penetrating the gastric mucosa, *H. pylori* releases proteins, polysaccharides, extracellular DNA (eDNA), and other molecules to create extracellular polymeric substances (EPS). These substances surround and adhere to each other by bacteria to form a biofilm. Unlike the planktonic form, the bacteria make biofilm structures that are resistant to harsh external environments, including exposure to antibiotics. Therefore, the formation of *H. pylori* biofilms may be a major cause of persistent infections, antibiotic resistance, and treatment failure [9, 10]. Notably, a biofilm of *H. pylori* contains virulence proteins such as AlpB, which plays an important role in supporting bacterial adhesion to gastric mucosal cells and in attaching bacterial cells together, the main steps in the biofilm formation process. *H. pylori* strains lacking AlpB will face difficulties in developing biofilms [10], [11].

**2.1.2. Stages of biofilm formation**

Biofilm formation of *H. pylori* is divided into four stages (Figure 1), including (1) attachment, (2) growth, (3) maturity, and (4) dispersal. At first, *H. pylori* adheres to the gastric mucosa. This process is driven by cilia, pili structures, and lipopolysaccharides (LPS), which are involved in the initial step of *H. pylori* infection. Next, the bacteria multiply and produce extracellular polymeric substances (EPS), promoting the association of bacteria with each other, and biofilm structure begins to form. The biofilm matures over 2-4 days and remains for a period. Then, when nutrients are depleted, and waste products accumulate to a certain threshold, the biofilm will disintegrate. After that, there may be a period of expansion of infection and biofilm formation at new locations [12].

Therefore, if the concentration or dosage of antibacterial agents is insufficient or if only agents that destroy biofilm are used, it will not only be ineffective in eradicating all bacteria but also break the biofilm. Hence, the dispersed bacterial flora can adhere to the gastric epithelium, further expanding the biofilm size and increasing the scope of infection. This may be the main reason for the phenomenon of biofilm size increasing instead of decreasing after using subinhibitory doses of antibiotics. Therefore, agents should not be used alone to only inhibit biofilm formation without antibacterial activity, antibiotics should be combined to improve treatment effectiveness [9].

**2.1.3. The role of *H. pylori* biofilm in antibiotic resistance**

Biofilm formation reduces the susceptibility of bacteria to antibiotics, making it difficult to eradicate *H. pylori*. Biofilm formation is considered a mechanism that helps *H. pylori* survive and infect the gastric mucosa for a long time. Studies have also found a correlation between biofilm formation and express increasing of many important virulence genes. Because the bacteria in biofilm are exposed to a number of harsh conditions, such as lack of nutrients, there can be an increase in mutation frequency and the emergence of mutant strains causing antibiotic resistance [11], [12]. According to research by Fauzia et al. (2020) in Indonesia, a higher antibiotic resistance rate on *H. pylori* strains can create strong biofilm in all tested antibiotics, and the difference was the most significant for clarithromycin ($p = 0.002$) [13].

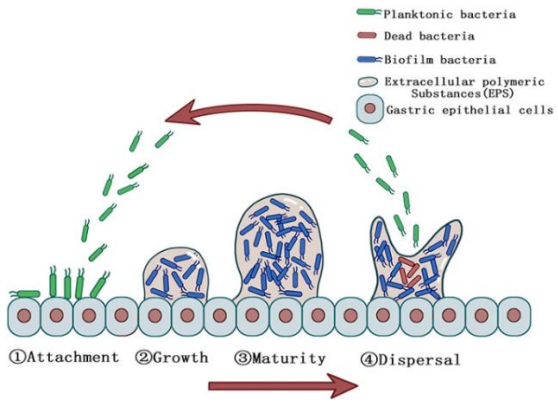


Figure 1. Steps of H. pylori biofilm formation
(Source: Hou, C., et al., 2022)

**2.1.4. Anti-biofilm agents**

Bacteria carrying biofilm structures can cause 10-1000 times more antibiotic resistance than bacteria in planktonic form. Therefore, developing agents aimed at impacting biofilm to potentially remove or disperse biofilm may be an effective strategy to reduce antibiotic resistance in *Helicobacter pylori*. Anti-biofilm agents can be considered complementary therapy or alternative therapy in *H. pylori* eradication regimens [14]. Pure compounds, extracts of natural origin, or some types of probiotics (*Lactobacillus sp*) are demonstrated to have anti-biofilm effects and can inhibit bacteria on *H. pylori* strains from being resistant to one antibiotic or multi-resistant, and data are presented in table 1. In the *in silico* and *in vitro* screening tests by Spiegel et al. (2021), the dioscin compound (a steroidal saponin) presents the most promising antibacterial, anti-biofilm formation activities and shows the synergistic activity when combined with three conventional antibiotics (clarithromycin, metronidazole, and levofloxacin) [15]. According to Xiao et al. (2022), allicin is highly sensitive to AlpB and is believed to have anti-biofilm effects by attaching to this protein [16].

**2.2. Anti-virulence activity**

**Another approach to eradicating *H. pylori* is targeting the bacteria's virulence factors. Inhibiting these factors can reduce the possibility of infection and increase the effectiveness of antibiotic therapies. Furthermore, the structure of many bacterial virulence factors has been clarified, so this treatment strategy has a number of advantages over conventional antibiotic therapies that can be mentioned as follows: (i) because it is not antibiotics so it does not affect the human gut microbiota; (ii) most virulence factors are extracellular molecules so inhibitors can be easily exposed to them [17].**

The mechanism of action of anti-virulence agents can be divided into two categories: (1) inhibition of a single virulence factor or (2) inhibition of processes involved in the regulation of *H. pylori* virulence such as impacting on the quorum sensing process (signal exchange pathway between bacterial cells), two-component regulatory system, the flagellum system, and the bacterial virulence secretion systems or the process of post-translational protein modifications because this process is related to protein maturation and affects the biological function of bacterial cells. Among these, the inhibition of quorum-sensing activity of *H. pylori* is most interesting [18],[19].

Table 1. Natural anti-biofilm agents targeting *H. pylori* infection

| Anti-biofilm agents | Models | Tested strain of *H. pylori* |
|---|---|---|
| *Chelidonium majus* and *Corydalis cheilanthifolia* extracts | *In vitro* | HP 8064 |
| *Atractylodes lancea* volatile oils | *In vitro* | NCTC 11637 |
| *Pistacia vera* L. oleoresin | *In vitro and in vivo* | 30 clinical strains, including multi-resistant strains |
| *Hibiscus rosa sinensis* Flower | *In vitro* | ATCC 43504, ATCC 51932 and 05 clinical strains (OX.22, OX.63, OX.64, OX.67, and OX.83) |
| *Acorus calamus* extract | *In vitro* | 40 clinical strains |
| *Vitex trifolia* extract | *In vitro* | 40 clinical strains |
| *Casearia sylvestris* leaf derivatives | *In vitro and in vivo* | ATCC 43504 |
| Dihydrotanshinon I (diterpenoid from *Salvia miltiorrhiza*) | *In vitro and in vivo* | ATCC 43504, HP G27, HP 26695, HP NSH57 and BHKS159 |
| Armeniaspirol A (from *Streptomyces armeniacus*) | *In vitro and in vivo* | HP G27, HP159, BHKS159 and 13 clinical strains |
| Curcumin | *In vitro* | ATCC43504, ATCC43526, ATCC51932 |
| Resveratrol (stilbenoids) | *In vitro* | ATCC 43629 and clinical strains |
| Myricetin (flavonoids) | *In vitro* | ATCC 700824, ATCC 51932 |
| Nimbolid (Limonoid from *Azadirachta indica*) | *In vitro* | ATCC 700684, HP G27, HP 26695, HPAG1, HP SS1, HP J99, HP 7.13 and USU101 |
| Carvacrol and Thymol (monoterpenoid) | *In vitro* | ATCC 43504 |
| Dioscin (steroidal saponin) | *In vitro* | HP J99 |
| *Lactobacillus plantarum* LN66 | *In vitro* | ATCC 43504 |
| *Lactobacillus salivarius* LN12 | *In vitro* | ATCC43504, HP SS1 and clinical strains |

### 2.2.1. Anti-VacA and anti-CagA agents

The key toxins of *H. pylori* are CagA and VacA, which play an important role in the infection process. The toxin CagA is encoded by the cag pathogenicity island (cagPAI) and translocated directly to gastric epithelial cells via the type IV secretion system (T4SS). Upon entering target cells, CagA activates NF-kB, a main system in immune regulation and inflammation, thereby increasing the secretion of proinflammatory cytokines (IL-8, TNF-α, and IL-1β). Meanwhile, VacA is secreted from *H. pylori*, which is a vacuolating cytotoxin causing apoptosis increase in host gastric mucosal cells. This toxin also increases the production of the inflammatory cytokine IL-8 by activating the MAPK p38 molecule through releasing intracellular $Ca^{2+}$ ions [11].

Phenolic compounds, especially flavonoids, are important representatives of the

compounds with anti-VacA and anti-CagA features. Data on the anti-VacA and anti-CagA properties of phenolics are presented in table 2. For instance, hesperetin is a natural flavanone found in several species of the *Citrus* genus, which presents potential anti-virulence effects. According to Kim et al. (2021), hesperetin inhibits the expression of CagA and VacA, reducing the translocation of CagA and VacA into AGS cells (a human gastric adenocarcinoma cell-line) in *in vitro* assay. The results are explained by the compound's ability to inhibit type IV secretion system (T4SS) and type V secretion system. In addition, hesperetin also has anti-*H. pylori* effects on different targets such as inhibiting the expression of important genes in the replication process (*dna*E, *dna*N, *dna*Q, *hol*B) and transcription (*rpo*A, *rpo*B, *rpo*D, *rpo*N). This compound also inhibits genes involved in bacterial motility (*flh*A, *fla*A, and *flg*E), adhesion factors (*Sab*A, *Alp*A, *Alp*B, *Hpa*A, and *Hop*Z), as well as inhibits the expression of urease [18], [20], [21].

Table 2. Anti-VacA and anti-CagA agents from phenolic compounds

| Classes | Compounds | Activities | Mechanisms |
|---|---|---|---|
| Flavonoid | Kaempferol | ◆Reduced secretion of IL-8, TNF-α, IL-1β.<br>◆Inhibition of VacA expression.<br>◆Prevents translocation of CagA and VacA into cells. | Inhibition of T4SS and T5SS systems |
| Flavonoid | Quercetin | ◆Reduced secretion of IL-8 | Reduced p38 MAPK activation |
| Flavonoid | Apigenin | ◆Reduced secretion of IL-8 | Inhibition of NF-kB activation |
| Flavonoid | Hesperidin | ◆Inhibition of VacA and CagA expression. | Inhibition of T4SS systems |
| Anthocyanin | Cyanidin-3-*O*-glucoside | ◆Inhibition of VacA and CagA expression. | Reduced expression of *sec*A protein. |

### 2.2.2. BabA and LPS inhibitors against *Helicobacter pylori*

Recently, adhesion factor BabA and lipopolysaccharide (LPS) molecules are also new targets in screening anti-*H. pylori* compounds. BabA is one of the most important adhesion factors, supporting bacteria in attaching to gastric mucosal cells. Meanwhile, LPS is an essential factor that helps *H. pylori* approach and adhere to the gastric mucosa through interaction with the host cell's galectin-3 receptor [22].

According to Gottesmann et al. (2020), the esterified rhamnogalacturonan molecules (polysaccharides class) isolated from *Abelmoschus esculentus* were demonstrated to inhibit the adhesion of *H. pylori* to AGS cells ($IC_{50}$ = 550.8 μg/mL) and inhibit BabA ($IC_{50}$ = 17.57 μg/mL) on *in vitro* model. An analysis of structure-activity relationship presented that rhamnogalacturonans with highly branched structure, low uronic acid content and high degree of esterification showed the strongest BabA inhibitory effect. These compounds also affected the attachment affinity of LPS to galectin-3 at different concentrations with an $IC_{50}$ value of 222 μg/mL [23].

### 2.2.3. Motility of *H.* pylori and anti-motility agents

The flagellum system is essential for the motility of *H. pylori*. The flagellum is composed of a basal body, a hook, and a filament. This filament contains two flagellin proteins, flaA and flaB molecules [11]; The hook is encoded by the flgE protein. Flagellin of filaments is a crucial part of the motility of *H. pylori*, necessary for bacterial entry or

maintenance of infection [24].

Piperine, an alkaloid from *Piper nigrum*, has the ability to inhibit genes involved in encoding flaA and flgE proteins of filament, leading to deterioration of flagella structure and inhibition of *H. pylori* motility [25]. Suerbaum et al. (2022) screened substances that inhibit the motility of *H. pylori* in an *in vitro* experiment and these substances are collectively called the antimotilin group. The results show that they are capable of blocking the flagellin biosynthesis process, directly inhibiting motility, and reducing the transcription process and the amount of flagellin protein. The new anti-motilin compound ($C_{12}H_{13}CIN_4OS$) belonging to the phenyl-pyrazolone framework displays the most potential data and is considered as a promising complementary therapy when combined with antibiotics in eradication treatment [26].

**2.2.4. *Helicobacter pylori* quorum sensing inhibitory effect**

The process of biofilm formation and bacterial toxins of *H. pylori* are controlled by the quorum sensing system, which is an intercellular communication process, through the chemoreceptor TlpB. Initially, Bakri (2021) screened *in silico* to search for inhibitors of the quorum sensing system of *H. pylori*. Among the 300 compounds tested, the 6 most potential compounds include β-carotene, β-amyrin, taraxasterol, bauerenol, taraxacin, and benzoyl peroxid. These inhibitors present high attachment affinity for TlpB, block the quorum sensing system of *H. pylori*, and present low toxicity in experimental models [27].

**2.3. Mucolytic agents**

The mucus layer that covers the gastric epithelium provides a neutral pH environment to shield and protect gastric cells. but at the same time creates a suitable environment for *H. pylori* bacteria to reside. This layer includes high-molecular-weight glycoproteins, called mucins, some lipids, and other small molecules and proteins [28]. Through disulfide bonds, the mucus layer has viscosity, which can reduce the diffusion rate and effectively decrease the penetration of antibiotics. Several studies have evaluated the potential of mucolytic activity as a complementary substance to antibiotic therapy. Among them, pronase, a proteolytic enzyme isolated from *Streptomyces griseus* (a gram-positive aerobic bacteria) in 1962, is known as a potential candidate with mucolytic effects and reducing mucus viscosity [17].

Gotoh et al. (2002), conducted a clinical trial on 135 patients in Japan with 2 groups: group A used triple eradication therapy including lansoprazole 30 mg x 1 time/day, amoxicillin 500 mg, and metronidazole 250 mg, 3 times/day; Group B received additional pronase with 18,000 units of tyrosine, combined with standard triple eradication treatment. The patient groups were followed for 2 weeks. As a result, pronase increased the eradication rate by about 15%, which is higher than that of conventional therapy. The thickness of the mucus layer in the pronase group was significantly thinner than the control group, especially in the antrum. Therefore, pronase may improve antibiotic distribution and/or make the gastric environment less favorable for bacteria [29].

However, another study by Bang et al. (2015) conducted on 116 patients in Korea, presented conflicting results with the above study. Similarly, patients were divided into 2 groups. While group A used triple eradication therapy, including PPI, amoxicillin 1000 mg, and clarithromycin 500 mg twice a day, group B combined pronase 20,000 units of tyrosine with the triple eradication therapy. In addition, patients in group B were also given 1 g of $NaHCO_3$ with the purpose of increasing gastric pH, contributing to the rise the effect of

pronase. Two groups of patients were treated for a period of 7 days. Consequently, the eradication rate in the group combined with pronase was significantly lower than in the group using conventional therapy alone, with rates of 56.1% and 76.4%, respectively (p = 0.029). Therefore, the scientists also give some explanations for the disagreements compared to Gotoh et al. (2002), which may be due to differences in dosage, duration of pharmaceutical use, method of administration of pronase, potential drug-drug interactions, and the observation period of the two studies [30].

Although more convincing data on the effectiveness of pronase in treatment are needed. Initially, these results also appreciate the potential role of agents with mucolytic activity in *H. pylori* eradication therapy.

### 2.4. Targeting vital proteins in the physiology of *H. pylori*

#### 2.4.1. Inhibitors of *H. pylori* Inosine-5′-monophosphate dehydrogenase

Inosine 5'-monophosphate dehydrogenase (IMPDH), is increasingly known as a potential target for the development of compounds to eradicate multidrug-resistant bacteria. This is an important enzyme in the biosynthesis of the guanine pathway, a nucleotide necessary for cell growth and development in general. Mechanistically, this enzyme oxidizes inosine 5′-monophosphate (IMP) to xanthosin 5′-monophosphate (XMP). Then, under the catalysis of GMP synthase, xanthosin 5′-monophosphate (XMP) is converted to guanosine 5′-monophosphate (GMP). The GMP molecule continues to create nucleotides to synthesize DNA and RNA of bacterial cells (Figure 2) [31], [32]. For cell growth and proliferation, guanine nucleotides are needed, and hence inhibiting the HpIMPDH leads to a decrease in the proliferation of bacterial cells.

However, IMPDH also exists in humans, most notably the type 2 IMPDH isoform (denoted as hIMPDH2). Inhibition of hIMPDH2 can lead to an impact on cell proliferation and impairment of intracellular signaling, which can cause adverse effects. Finding compounds that selectively inhibit the IMPDH of *H. pylori* and have little/no interaction with hIMPDH2 is necessary to avoid the risks of toxicity [32, 33]. Initially, some flavonoid and phthalide framework compounds were reported to have potential effects when demonstrating the ability to selectively inhibit the IMPDH of *H. pylori* bacteria (Hp-IMPDH inhibitors) [34].


Figure 2. Mechanism of Hp-IMPDH inhibitors
(Source: Juvale, K. et al, 2019)

#### 2.4.2. HsrA inhibitors

HsrA (homeostatic regulator) is an essential protein for the viability of *H. pylori* because it attaches to bacterial DNA and regulates various functions, including homeostasis, metabolic synchronization, nitrogen metabolism, regulation of virulence factors, and cell division. Notably, this molecule is not present in humans, so HsrA becomes a promising target for anti-*H. pylori* therapy [19]. González et al. (2019) conducted an *in vitro* screening

of several compounds capable of attaching and inhibiting *H. pylori* HsrA. Among 1,120 compounds surveyed, there are 07 natural compounds belonging to the flavonoid framework, including chrysin, apigenin, luteolin, hesperidin, kaempferol, quercetin, and myricetin. These compounds inhibit the formation of binding between HsrA and DNA, thereby causing *H. pylori* inhibitory activity [35].

## III. CONCLUSION

In these days, antibiotic resistance associated with *H. pylori* has become a major challenge in clinical practice. Drugs derived from medicinal plants and their constituents with new mechanisms such as anti-biofilm, anti-virulence, mucolytic agents, inosine-5-monophosphate dehydrogenase, and HsrA inhibitors against *H. pylori* show promise as prospective compounds for complementary therapy that aims to increase the *H. pylori* eradication rate, especially on multidrug-resistant bacteria. Natural ingredients can provide valuable insights for the discovery of new antimicrobial drugs. This article aims to expand the range of treatment options available for the eradication of *H. pylori* and provide insights into the development of natural products. Finally, even more research studies are required to elucidate the mechanisms of action of these molecules, their synergisms, antagonisms, and other pharmacological aspects.

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