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REVIEW ARTICLE

Effects of plant essential oils and their constituents on *Helicobacter pylori*: A review

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Abstract

Essential oils (EOs) obtained from different medicinal and aromatic plant families by steam distillation have been used in the pharmaceutical, food, and fragrance industries. The plant EOs and their broad diversity of chemical components have attracted researchers worldwide due to their human health benefits and antibacterial properties, especially their treatment of *Helicobacter pylori* infection. Since *H. pylori* has been known to be responsible for various gastric and duodenal diseases such as atrophic gastritis, peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma, several combination antibiotic therapies have been increasingly used to enhance the eradication rate of the bacterial infection. However, in the last decades, the efficacy of the therapies has decreased significantly due to widespread emergence of multidrug resistant strains of *H. pylori*. In addition, side-effects from commonly used antibiotics and recurrence of the bacterial infection have drawn public health concern globally.

Therefore, this review focuses on *in vitro* effects of plant EOs and their bioactive constituents on the growth, cell morphology and integrity, biofilm formation, motility, adhesion, and urease activity of *H. pylori*. Their inhibitory effects on expression of genes necessary for growth and virulence factor productions of the bacterial pathogen are also discussed. Further *in vivo* and clinical evaluations are required so that plant EOs and their bioactive constituents can be possibly applicable in pharmacy or as adjuvants to the current therapies of *H. pylori* infection.

Keywords

Essential oil; chemical constituent; antibacterial activity; inhibition of gene expression; mode of action; *Helicobacter pylori*

Introduction

Folk medicinal and aromatic plants are a rich source of essential oils (EOs) as complex mixtures of mono and sesquiterpene hydrocarbons, and their oxygenated derivatives such as aldehydes, ethers, alcohols, esters, ketones, phenols and oxides (1). They exhibit a plethora of health benefits and are relatively safe as no serious side effects and often act at multiple and novel target sites, thereby reducing the risk of resistance development (2). A number of medicinal plant extracts and EOs traditionally used for treatment of gastrointestinal disorder, have been reported to display gastro-protective effect (3-6) and anti-*Helicobacter pylori* activity (7-10). Therefore, plant EOs and their chemical constituents have been suggested as safe alternative or

combination agents with antibiotics for treatment of bacteria with no risk of resistance developments, especially in treatment of antibiotic-resistant strains of *H. pylori* (11-14).

Helicobacter pylori is a Gram-negative microaerophilic spiral bacterium successfully isolated from human gastric biopsy specimens by Warren and Marshall (15, 16). The bacterial pathogen can produce several virulence factors for its invasion and persistent colonization in epithelial cells of the human stomach (17). The bacterium is considered to be the most common cause of gastritis worldwide and strongly associated with peptic ulcer and gastric carcinogenesis. More than half of the world's population was estimated to be infected with *H. pylori* and the prevalence of infection tends to increase with age and low socioeconomic status (18). It was reported that acute and chronic gastritis was mostly detected in *H. pylori*-positive patients. If left untreated, the chronic gastritis could lead to more severe conditions, including atrophic gastritis (5%), peptic ulcer disease (10%), low-grade gastric mucosa -associated lymphoid tissue (MALT)-lymphoma (< 1%), and thereafter gastric cancer (1%) (19). Combination of antibiotic therapies have been prescribed and greatly contributed to their success in controlling the bacterial infection (19). However, serious side-effects of the frequently used antibiotics and increasing appearance of drug-resistant strains of *H. pylori* have led to the failure of the therapies (18, 20).

Therefore, *in vitro* and *in vivo* antibacterial activities of plant EOs and their components toward *H. pylori* are reviewed and discussed in the present article. Mode of action of the EOs and bioactive components on *H. pylori* is also highlighted.

Growth-inhibiting effect of EOs and their constituents on H. pylori

EOs from various plant species are complex mixtures of volatile constituents, which jointly or independently contribute to the growth inhibitory effect on both susceptible and resistant strains of *H. pylori,* and pose less or no risk of emergence of resistant strains (14, 21, 22).

Table 1 and Table 2 show plant EOs with their major components, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the EOs and constituents against *H. pylori*, respectively. EOs from different plant species presented in Table 1 exhibited significantly different antibacterial activities against *H. pylori*. The high antimicrobial activity of some plant EOs could be attributed to their major components. The EOs from *Thymus* L. species characterized by high amounts of monoterpene phenols (thymol and/or carvacrol) showed potent antibacterial effects against *H. pylori*. EO from *T. capitatus* (L.) Hoffmanns. & Link displayed the highest growth inhibitory effect and bactericidal activities with MIC (MBC) values of

Table 1. Essential oils with main components and minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of the EOs against *H. pylori*

Essential oil	Part	Main components (%)	MIC (MBC) (µg/mL)	References
Thymus capitatus (L.) Hoffmanns. & Link	Aerial parts	thymol (47.2), p-cymene (15.1), γ-terpinene (10.0)	0.25 (0.5)	(23)
Thymus carmanicus Jalas	Aerial parts	carvacrol (68.9)	14.5	(24)
Thymus zygis L.	Aerial parts	carvacrol (36.7), p-cymene (28.3), γ-terpinene (18.4),	50	(28)
Oliveria decumbens Vent.	Aerial parts	thymol (38.8), carvacrol (36.3)	20.4	(27)
Origanum vulgare L.	Aerial parts	carvacrol (67.7), p-cymene (14.6)	31.3 (31.3)	(26)
Satureja montana L.	Leaf	carvacrol (36.5), p-cymene (12.0)	(40)	(47)
Spondias pinnata (L. f.) Kurz	Leaf	β-caryophyllene (49.9), α-terpineol (27.0)	1.95	(40)
Hypericum perforatum L.	Aerial parts	β-caryophyllene (19,1), germacrene-D (13,2), α-pinene (12,6)	4	(41)
Taxodium distichum (L.) Rich.	Leaf	α-pinene (83.1)	7.8	(43)
Cannabis sativa L.	Aerial parts	β-caryophyllene (28.0), caryophyllene oxide (15.0), humulene (13.0), β-myrcene (11.0)	8 (8)	(42)
Apium nodiflorum (L.) Lag.	Aerial parts	limonene (27.7), p-cymene (23.1), myristicine (18.5)	12.5	(32)
Cymbopogon schoenanthus (L.) Spreng.	Aerial parts	geranial (42.8), neral (32.6)	15.6 (15.6)	(26)
Cymbopagon citratus (DC.) Stapf	Aerial parts	citral (72.9)	40	(7)
Daucus carota L.	Seed	carotol (34.4), α-pinene (13.3), geranyl acetate (10.4)	(20)	(7)
Juniperus virginiana L.	Bark	α-cedrene (22.9), thujopsene (21.8), cedrol (15.1)	15.6 (62.5)	(26)
Cinnamomum glanduliferum (Wall.) Meisn.	Bark	1,8-cineole (65.9)	31.3	(34)
Cinnamomum zeylanicum Blume	Bark	cinnamaldehyde (41.8), eugenol (23.3)	(40)	(7)
Pachira aquatica Aubl.	Leaf	palmitic acid methyl ester (21.1)	31.3	(47)
Melaleuca alternifolia (Maiden & Betche) Cheel	Leaf and twig	terpinen-4-ol (39.6), γ-terpinene (19.3)	62.5 (62.5)	(26)
Citrus paradisi Macfad.	Peel	limonene (92.3)	(100)	(7)

Citrus limon (L.) Osbeck	Peel	limonene (58.1), β-pinene (17.0)	125 (250)	(26)
Plinia cerrocampanensis Barrie	Leaf	α-bisabolol (42.8), bisabolol oxide B (10.3)	62.5 (62.5)	(46)
Pinus silvestris L.	Needles	α-pinene (31.1), β-pinene (20.4), 3-carene (15.0), limonene (12.1)	125 (125)	(26)
Abies alba Mill.	Needles	bornyl acetate (53.2), α-pinene (15.5)	125 (250)	(26)
Nepeta argolica Bory & Chaub. ssp. *dirphya*	Aerial parts	4aα,7α,7aα-nepetalactone (58.1), 4aα,7α,7aα-nepetalactone (17.0)	128	(39)
Paeonia lactiflora Pall.	Root	paeonol (20.3), myrtenol (11.3), myrtenal (10.0)	160 (310)	(49)

0.25 (0.5) µg/mL (23), followed by those of *T. carmanicus* Jalas EO with a MIC of 14.5 µg/mL (24) and *T. vulgaris* L. EO characterized by thymol (45.9%) and p-cymene (13.4%) as major components (25) displayed MIC (MBC) of 15.6 (16.5) µg/mL (26). Carvacrol and/or thymol rich EOs of other genera such as *Oliveria decumbens* Vent. (27), *Origanum vulgare* L. (26), and *Satureja montana* L. (7) were also found to possess remarkable anti-*H. pylori* effects with MIC and/or (MBC) values of 20.4, 31.3 (31.3) and (40) µg/mL, respectively, while the EO of *T. zygis* L. gave a higher MIC value (50 µg/mL) (28). In the EO of *O. majorana* Vent., terpinen-4-ol (23.3%), γ-terpinene (15.9%), α-terpinene (11%) were present as main compounds (29). The *O. majorana* EO was shown to display lower anti-*H. pylori* activity (MIC (MBC) of 62.5 (250) µg/mL) (26) as compared with its counterpart of *O. vulgare* EO (Table 1). These results show that the presence of high content of the monoterpene phenols contributed to the antibacterial activity of these EOs.

As reported in Table 2, cinamaldehyde and eugenol exerted the highest growth inhibitory activity against *H. pylori* with MIC value of 1 µg/mL (21), followed by those of thymol with MIC (MBC) of 7.8 (31.3) µg/mL (26), patchouli alcohol with MIC (MBC) of 12.5 (25) µg/mL (22, 30, 31), menthol with MIC (MBC) of 15.6 (31.3) µg/mL (26), and carvacrol with MIC of 20 µg/mL (24). The EOs of *T. capitatus* and *T. carmanicus* had stronger anti-*H. pylori* activities than thymol and carvacrol, respectively, while the antibacterial activity of thymol and carvacrol were stronger than those of the *T. vulgaris* and *T. zygis* EOs, respectively. The EO of *O. decumbens* contained both thymol (38.8%) and carvacrol (36.3%) (27), but the anti-*H. pylori* activity of *O. decumbens* EO showed was much lower than those of *T. capitatus*, *T. carmanicus* and *T. vulgaris* EOs which contain only thymol or carvacrol (Table 1). Similarly, *Apium nodiflorum* (L.) Lag. EO containing limonene (27.7%), p-cymene (23.1%), myristicine (18.5%) was shown to be a strong anti-*H. pylori* agent with MIC value of 12.5 µg/mL (32), which exhibited significantly stronger antibacterial effect than limonene (MIC of 75 µg/mL) (5). Therefore, p-cymene, g-terpinene, myristicine and/or minor components in the EO may also contribute to the anti-*H. pylori* activity.

Ali *et al.,* (21) reported that cinnamaldehyde and eugenol interestingly displayed a strong growth inhibitory activity against *H. pylori* with MIC value of 1 µg/mL. Stem bark EO of *Cinnamomum zeylanicum* Blume possesses both cinnamaldehyde (41.8%) and eugenol (23.3%), so it exhibited a significant bactericidal activity with MBC value of 40 µg/mL against *H. pylori* (7). According to Bergonzelli *et al.,* (7), isoeugenol (with MBC value of 40 µg/mL) has a strong bactericidal activity than eugenol (with MBC value of 100 µg/mL). EOs from *Ocimum basilicum* L. and *Pelargonium odoratissimum* (L.) L'Hér. respectively contained methyl chavicol (83.5%) (14) and methyl eugenol (96.8%) (33) as a single main component and both the EOs exhibit-

Table 2. Antibacterial activity of EO constituents against *H. pylori*

Constituent	MIC (MBC) (µg/mL)	References
Cinnamaldehyde	1	(21)
Eugenol	1	(21)
Thymol	7.8 (31.3)	(26)
Patchouli alcohol	12.5 (25)	(22, 30, 31)
Menthol	15.6 (31.3)	(26)
Carvacrol	20	(24)
Bisabolol	31.3 (31.3)	(26)
α-Terpinolene	40 (40)	(49)
Citral	(40)	(7)
Isoeugenol	(40)	(7)
Nerol	(40)	(7)
4aα,7α,7aβ-nepetalactone	64	(39)
4aα,7α,7aα-nepetalactone	128	(39)
Limonene	75	(5)
α-pinene	(100)	(7)
β-pinene	(100)	(7)
Citronellal	(100)	(7)
Geraniol	(100)	(7)
Linalool	(100)	(7)
(–)-Borneol	80 (160)	(49)
(–)-Perilla alcohol	80 (160)	(49)
(1R)-(–)-Myrtenol	80 (160)	(49)
(1S,2S,5S)-(–)-Myrtanol	80 (160)	(49)
1,8-Cineole	128	(39)
(–)-Perillaldehyde	160 (310)	(49)
(±)-Lavandulol	160 (310)	(49)
(1R)-(–)-Myrtenal	160 (310)	(49)
Paeonol	160 (620)	(49)
Cuminaldehyde	310 (310)	(49)
β-Myrcene	500	(3)
(E)-Anethole	620 (1250)	(49)
Limonene oxide	620 (620)	(49)
β-Caryophyllene	1000	(44, 45)

ed anti-*H. pylori* with significantly higher MIC values (15.6 and 62.5 µg/mL, respectively) (26) as compared with eugenol. The results indicated that methyl chavicol (known as estragole) and methyl eugenol had lower antibacterial activity than those of isoeugenol and eugenol against *H. pylori*. Therefore, various proportions of phenylpropanoids in plant EOs such as cinnamaldehyde, eugenol, isoeugenol, methyl eugenol and estragole could have contributed to the growth inhibitory and bactericidal activities against the pathogen.

EOs from other *Cinnamomum* L. species rich in 1,8 -cineole also contributed to the anti-*H. pylori* activity. The EO of *C. glanduliferum* (Wall.) Meisn. dominated 1,8-cineole (65.9%), showed a strong anti-*H. pylori* activity with MIC of 31.3 µg/mL) (34) whereas the EO of *C. camphora* (L.) J. Presl dominated by camphor had a low antibacterial activity against *H. pylori* with MIC (MBC) of 125 (125) µg/mL (26) (Table 1). The compound 1,8-cineole (or eucalyptol) is also known to promote ulcer healing and act as a gastro protective agent (35). It has been found in several plant EOs (Table 1) such as *Melaleuca quinquenervia* (Cav.) S.T.Blake (50.2%), *M. leucadendra* (L.) L. (60.8%) (36) and *Eucalyptus globulus* Labill. (58.5%) (14) which also displayed a potent antibacterial activity against *H. pylori* with MICs of 31.3–46.4 µg/mL (26, 37). The EOs of *Rosmarinus officinalis* L. (29), *Salvia officinalis* L. and *Lavandula angustifolia* Mill. (38) containing both 1,8-cineole and camphor as major components were reported to have lower antibacterial activity against *H. pylori* (7, 26). Surprisingly, both the compounds (1,8-cineole and camphor) have been reported to induce lower anti-*H. pylori* activity than these EOs (7, 39). Similarly, several EOs are characterized by high percentage of β-caryophyllene which is found in *Spondias pinnata* (L. f.) Kurz (49.9%) (40), *Hypericum perforatum* L. (19.1%) (41), and *Cannabis sativa* L. (28%) (42). And α-pinene is found in *Taxodium distichum* (L.) Rich. (83.1%) (43) which also displayed the highest growth inhibitory activities against *H. pylori* with MIC values ranging from 1.95 to 8 µg/mL (Table 1). However, α- and b-pinene (MBC of 100 µg/mL) (5, 7), β-myrcene (MIC of 500 µg/mL) (3) and β-caryophyllene (MIC of 1000 µg/mL) (44, 45) (Table 2) exhibited significantly lower anti-*H. pylori* effect than the EOs suggesting that other major and/or minor components have also critically contributed to the antibacterial activity of the EOs of *S. pinnata*, *H. perforatum*, *C. sativa*, and *T. distichum*.

Unlike the bicyclic sesquiterpene β-caryophyllene, bisabolol is a monocyclic sesquiterpene alcohol that possesses a potent growth inhibitory and bactericidal effect on *H. pylori* (26). The EO from *Plinia cerrocampanensis* Barrie dominated by α-bisabolol (42.8%) and bisabolol oxide B (10.3%) was also shown to exert an antibacterial action against *H. pylori* (46). In addition, some sesquiterpenes and sesquiterpene alcohol such as α-cedrene (22.9%), thujopsene (21.8%) and cedrol (15.1%) were identified as main components of *Juniperus virginiana* L. EO which possesses a potent anti-*H. pylori* effect (26) (Table 1). The high content of the sesquiterpenes as well as oxygenated sesquiterpenes could participate in the antibacterial action of the EOs against the bacterium. The EO from leaf of *Pachira aquatica* Aubl. characterized by high content of palmitic acid methyl ester (21.1%) were shown to have effectiveness against *H. pylori* infection (47) (Table 1). And the potent inhibitory effect of the EO was suggested to be attributed to its high content of many sesquiterpenes and oxygenated sesquiterpenes, which constituted about 40% of the EO, such as α-cedrene, cedr-8-ene, α-gurjunene, hexahydrofarnesyl acetone, t-elemene, trans-nerolidol and β-ionene (47). Nevertheless, there is no report on antibacterial activity of the sesquiterpenes and sesquiterpenoids against *H. pylori.* In addition, free fatty acids including linolenic acid have been known to exert noteworthy antibacterial property against *H. pylori* (48) but there is no information on the anti-*H. pylori* activity of fatty acid methyl ester, including palmitic acid methyl ester.

Citral (a mixture of stereoisomers neral and geranial) has been reported to be a bactericidal agent against *H. pylori* with MBC of 40 µg/mL (7) (Table 2). EOs of *Cymbopogon schoenanthus* (L.) Spreng. and *C. citratus* (DC.) Stapf were found to display the antibacterial activity with MIC (MBC) of 15.6 (15.6) µg/mL (26) and MIC of 40 µg/mL (7), respectively (Table 1). Citral was present up to 70% (7, 26) and could be the main active component in both of these essential oils. In *Lippia citriodora* (Palau) Kunth EO, neral and geranial account for only 30.5% of the EO (14), but the bactericidal effect of the EO was similar to that of citral (7). Menthol, a monoterpene alcohol, obtained from various species of genus *Mentha* L. was reported to have growth inhibitory and bactericidal properties against *H. pylori* with MIC (MBC) value of 15.6 (31.3) µg/mL) (26) (Table 2). In the EO of *Mentha piperita* L., menthone, menthol, isomenthone were found to be major components with percent areas of 41.1, 19.1, and 11.9, respectively (29). However, the anti-*H. pylori* activity of the *M. piperita* EO (MIC (MBC) value of 62.5 (500) µg/mL) was significantly lower than that of menthol (26) (Table 2). It was reported that citral, isoeugenol, a-terpinolene and nerol had the same bactericidal potency (MBC of 40 µg/mL) (7, 49), but several other monoterpenes and monoterpenoids such as nepetalactones, limonene, limonene oxide, citronellal, geraniol, linalool, borneol, perilla alcohol, myrtenol, myrtanol, myrtenal, lavandulol, cuminaldehyde, phenolic paeonol, and phenylpropanoid anethole exhibited lower antibacterial activity against *H. pylori* (5, 7, 39, 49) (Table 2). The peel EOs of two *Citrus* L. species, *C. paradisi* Macfad. and *C. limon* (L.) Osbeck EOs dominated by 92.3 and 58.1% limonene, respectively (7, 26) (Table 1), showed lower anti-*H. pylori* action than the compound limonene (5) (Table 2), while the flower EO of *C. aurantium* L. was reported to give significantly stronger antibacterial activity against *H. pylori* with MIC (MBC) of 31.3 (62.5) µg/mL (26) than the compound limonene. The presence of the anti- *H. pylori* components and synergic or antagonic interactions of other volatile components may have enhanced or reduced the antimicrobial activity of plant EOs. Moreover, difference in volatile constituents and their area percentages obviously influenced the antimicrobial effect of plant EOs.

Growth inhibitory activity of EOs and their compounds against *H. pylori* was dependent on exposure time and concentration and pH of test medium. *C. citratus* and *L. citriodora* EOs were shown to exhibit bactericidal activity against *H. pylori* after 12 hours of incubation at 0.02% (v/v) and the bactericidal activity of *C. citratus* EO was at 0.01% (v/v) after 48 hours of treatment (14). Citral rich hydrosol from steam distillation of *Litsea cubeba* (Lour.) Pers., at 30% of the hydrosol in the bacterial cultures (v/v), was shown to cause a sharp decrease the density of *H. pylori* from 5.92 to 2.61 Log_{10}CFU/mL after 18 hours and had complete bactericidal activity after 24 hours of treatments (50). *Atractylodes lancea* (Thunb.) DC. EO had a low anti-*H. pylori* effect (MIC of 7500 µg/mL) and inhibited the bacterial growth at 1/8 × MIC after 48 hours but the inhibition disappeared after 72 hours of incubation (51). At 2 µg/mL, eugenol and cinnamaldehyde could inhibit the growth of all the 30 *H. pylori* strains tested in the 9th and 12th hours of incubation, respectively, and the bactericidal activity of both the compounds was shown to increase in acidic medium (pH = 4.0) (21). The bactericidal activity of EOs from *C. citratus*, *Daucus carota* L. and *C. paradisi* at various concentrations were also reported to significantly increase at pH 4.0 compared to those at pH 5–7.4 (7, 14). Patchouli alcohol (Table 2), a major active component of *Pogostemon cablin* (Blanco) Benth. EO, at 5 × MIC produced bactericidal activity against *H. pylori* NCTC 11637 at pH 5.3 and 7, and *H. pylori* SS1 at pH 7 after 15 min of incubation (22). In addition, mixtures of different EOs were effective in helping to enhance antibacterial activity towards *H. pylori*. Binary and tertiary mixtures of *Satureja hortensis* L. and *O. vulgare* EOs decreased 2–4 × MIC values in comparison with a single EO treatment (29). Therefore, the inhibitory activities of the essential oils result from the complex interactions between different plant EOs and among many constituents, which may result in additive, synergistic or antagonistic effects against the organism.

Poor water-solubility and high volatility are major drawbacks of plant EOs, which adversely influence their antibacterial activity and limit their application (52, 53). Combination of *T. capitatus* EO with monolaurin derived from lauric acid and glycerin, was found to displayed higher antibacterial activity against *H. pylori* (MIC = 31 µg/mL) than the *T. capitatus* EO alone (500 µg/mL) (54). Liposomal linolenic acid was reported to have effectiveness against *H. pylori* (MBC of 200 µg/mL), whereas liposomal stearic acid displayed a significant lower anti-*H. pylori* activity (MBC of 1000 µg/mL), and liposomal oleic acid did not exhibit an antibacterial effect against *H. pylori* (48). Liposomal linolenic acid has been shown to promote permeability of outer membrane of *H. pylori*, resulting in bacteria cell death (48). Therefore, the high content of fatty acids and/or fatty acid methyl ester in plant EOs could enhance the antibacterial activity of the EOs against the pathogen. Moreover, the antibacterial activity of *C. zeylanicum* EO nanoemulsion was significantly improved against isolated strains of *H. pylori* (MBC value of 60–470 µg/mL) compared to that of the cinnamon oil alone (MBC value of 140–2340 µg/mL) (52). Eugenol, vanillin, carvacrol, and thymol immobilized on the amorphous silica microparticles were also reported to inhibit *H. pylori* growth and lead to the cell death at MBC between 25–50 µg/mL (53). Mode of action of plant EOs and their constituents on *H. pylori* was presented in Fig. 1.

Effect on H. pylori urease activity

For successful survival in acidity and colonization of the gastric mucosa, *H. pylori* produces urease to yield copious amounts of ammonia through urea hydrolysis to neutralize the pH of the bacterial surroundings (17). Hence, inhibition of *H. pylori* urease offers a great potential for controlling the bacterial infection (pathway 4 in Fig. 1). Plant EOs and their constituents were demonstrated to exhibit strong inhibition effect on urease of *H. pylori*. Patchouli alcohol

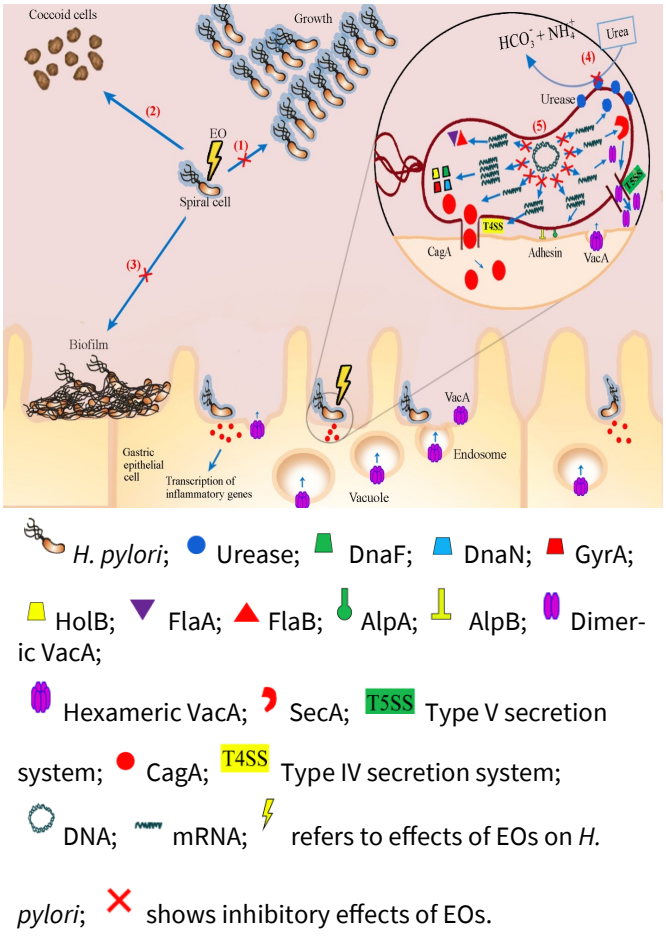

Fig. 1. Mechanisms of action of EOs and their compounds against *H. pylori*. EOs and their compounds inhibit the growth of *H. pylori* (**1**), causing the morphological conversion from spiral to coccoid forms (**2**), suppressing the biofilm formation (**3**) and urease catalytic activity (**4**). EOs and their compounds suppress expression of genes necessary for growth (*dna*E, *dna*N, *hol*B, and *gyr*A), motility (*fla*A and *fla*B), adhesion (*alp*A and *alp*B), production and translocation of virulence factors (Urease, VacA, CagA, SecA, and T4SS) (**5**).

was shown to prevent the growth of *H. pylori* by inhibition of the bacterial urease (Table 3) and 5 times stronger than the positive control acetohydroxamic acid (31). Another study indicated that patchouli alcohol at 25 and 50 µM also reduced the survival rate of *H. pylori* by inhibiting the acid resistance ability of *H. pylori* (30) (Table 3). The EOs of *J. virginiana* and *Pinus silvestris* L. exhibited the highest inhibitory effect on *H. pylori* urease activity with IC_{50} values of 5.3 and 18.4 µg/mL, respectively, while *C. limon, Abies alba* Mill., *Melaleuca alternifolia* (Maiden & Betche) Cheel *and C. schoenanthus* EOs had moderate inhibitory activity

on the enzyme (IC_{50}, 35.6–67.1 µg/mL), and low or no activity (> 200 µg/mL) was found with *O. vulgare* and *T. vulgaris EOs* (Table 3) despite their potent growth-inhibiting effects on *H. pylori* (26) (Table 1). This indicates *O. vulgare* and *T. vulgaris* EOs possess completely different mode of action against the growth of *H. pylori*. It was reported in Table 2 that α- and β-pinene, β-myrcene, and β-caryophyllene had low growth-inhibiting effect on *H. pylori*. Nonetheless, b-pinene and β-myrcene were indicated to have gastroprotective effect (3, 5, 7), while β-caryophyllene can reduce colonization and inhibit and/or decrease expressions of *H. pylori* virulent genes (44, 45) (Table 3). Generally, the antimicrobial activity of some plant EOs and their components involve several sites of action at the cellular level. Possible mode of action is the inhibition of protease production, therefore affects the growth ability of *H. pylori* in acidic environment of the stomach and stops the toxin production and motility of *H. pylori*.

Effect on morphology, membrane integrity and motility capacity of H. pylori

Both spiral shape and flagella of *H. pylori* are crucial for successful colonization of the stomach since these properties allow the pathogen to cross the mucus layer and reach the epithelium (17). Pathway 2 in Fig. 1 indicated adverse effect on the morphology and flagella of *H. pylori*, providing an important aspect for using natural products to treat the bacterial infection. Root EO of *Paeonia lactiflora* Pall. and its components (Table 1) could cause an abnormal change in morphology of *H. pylori* (Table 3) in which most of spiral cells transformed to irregular coccoid with rough surface and no flagella (49). Eugenol, cinnamaldehyde and patchouli alcohol (Table 3) could induce a considerable conversion of *H. pylori* from spiral to coccoid forms and cause damages to the cell wall and cytoplasmic membrane (21, 22). In addition, patchouli alcohol was also found to reduce significantly the motility of *H. pylori* in the swarm agar plate (Table 3) by inhibiting the formation of *H. pylori* flagella and making the flagella shorter than the originals (22). No spiral cells of *H. pylori* were observed after 24 hours of incubation with eugenol, vanillin and carvacrol immobilized on the amorphous silica microparticles, while coccoid and short bacilli forms were visualized with eugenol or vanillin treatment alone and with carvacrol treatment only, respectively (53). These bioactive components could attach to the cell surface and irreversibly disturb the structural integrity of cell membrane that would detrimentally influence the cell morphology and metabolism resulting in cytoplasmic losses, leakage of ions, loss of energy substrates, and lead to cell death (53).

Table 3. Urease inhibitory and other activities of plant EOs and constituents against *H. pylori*

Essential oil or component	Urease inhibition (IC_{50}, µg/mL)	Other activities	References
Juniperus virginiana L.	5.3		
Pinus silvestris L.	18.4		
Citrus limon (L.) Osbeck	35.6		
Abies alba Mill.	37.9	Antioxidative activity	(26)
Melaleuca alternifolia (Maiden & Betche) Cheel	39.1		
Cymbopogon schoenanthus (L.) Spreng.	67.1		
Origanum vulgare L.	208.3		
Thymus vulgaris L.	248.7	Antioxidative activity	(7), (26)
Paeonia lactiflora Pall.		Effect on morphology	(49)
Daucus carota L.		Effect on colonization	(7)
Croton cajucara Benth.		Gastroprotective effect	(6)
Pistacia atlantica Desf.		Gastroprotective effect	(4)
Atractylodes lancea (Thunb.) DC.		Anti-biofilm and decrease Cag A and IL-8	(51)
Patchouli alcohol	593.7	Effect on colonization and inflammation, adhesive capacity, motility, flagellar formation and morphology; decrease adhesion and motility-related gene expression (*alpA, alpB, flaA and flaB),* gene expressions of *ure*B, *ure*E, *ure*I and *nix*A; and reduce protein expression of UreB;	(22, 30, 31)
Cinnamaldehyde		Effect on morphology	(21)
Eugenol		Effect on morphology	(21)
Limonene		Gastroprotective effect	(5)
β-Pinene		Gastroprotective effect	(5, 7)
β-Myrcene		Gastroprotective activity and antioxidant effect	(3)
β-Caryophyllene		Reduce colonization, expressions of CagA, T4SS, SecA, and VacA; and *dnaE, dnaN, holB*, and *gyrA*.	(44, 45)

Effect on adhesive capacity and biofilm formation

H. pylori can utilize its adhesins, bacterial cell-surface proteins, to attach to membrane-associated receptors on the mucus-secreting gastric epithelial cells, leading to successful colonization and persistent infection (55). In addition, biofilms, aggregates of microorganisms encased in an extracellular polymeric substance, protect the microbial community from immune cells and antimicrobial agents (56). Therefore, *H. pylori* could be eliminated by inhibiting its biofilm formation (showed in pathway 3 in Fig. 1) and adhesive capacity (showed in pathway 5 in Fig. 1). The EO of *O. minutiflorum* O.Schwarz & P.H.Davis was proven to exhibit a considerable bactericidal effect on *H. pylori* adhered to and invaded human gastric cells (AGS), and could reduce more than 80% of the bacterial cells to adhere to the AGS cells, compared to the control treatments (57). The adhesion of *H. pylori* to gastric epithelial (GES-1) cells was also decreased remarkably as the bacterial cells were pretreated with patchouli alcohol or cocultured with GES-1 and patchouli alcohol at 5, 10, and 20 µg/mL for 1 h (22). Plant EOs have been demonstrated to have anti-biofilm activity against both Gram negative and positive bacteria, such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Chromobacterium violaceum* (58, 59). However, there is less information reported on anti-biofilm activity of plant EOs toward *H. pylori*, except for the EO of *A. lancea*. found to reduce biofilm formation of *H. pylori* at sub-MIC concentration (51) (Table 1). Further studies need to be carried out to clarify the anti-biofilm activity of EOs and their constituents against *H. pylori.*

Effect on gene expression of H. pylori

H. pylori can persistently colonize in the human stomach and induce severe gastric diseases by producing a unique set of virulence factors. Plant EOs and their compositions have been proven to be potential agents for suppressing expression of several virulence genes, firstly related to its survival and growth in the gastric lumen, then associated with its active motility for successful colonization of the mucus layer, and finally involved in virulence factors that cause direct epithelial damage and inflammation (as shown in pathway 5 in Fig. 1).

First of all, for the expression of catalytically active urease, *H. pylori* possesses two *ureA* and *ureB* genes (that encode for the two subunits of urease, an apoenzyme), a *nixA* (that encodes a high-affinity Ni^{2+} transporter), and five accessory genes such as *ureE, ureF, ureG*, and *ureH* (that encode accessory proteins necessary for Ni^{2+} insertion into the apoenzyme) and *ureI* (that encodes an acid-activated urea transporter crucial for acid resistance of *H. pylori*) (60). Patchouli alcohol at both concentrations of 25 and 50 µM were reported to able to down regulate the gene expression levels of *ureB*, *ureE*, *ureI*, and *nixA* and reduce the protein expression level of UreB, thereby inhibiting intracellular urease activity and acid resistance of *H. pylori* (30).

Next, DNA gyrase encoded by two genes (*GyrA* and *GyrB*) and DNA polymerase III encoded by six genes (*dnaE, dnaQ, dnaN, dnaX, holA,* and *holB*) (Fig. 1) are essential for the bacterial replication (61). β-caryophyllene was shown to possess inhibitory activity against the expressions of replication genes of *H. pylori* such as *CagA*, *VacA*, *SecA*, *T4SS*, *dnaE*, *dnaN*, *holB*, and *gyrA* (44, 45) (Table 3). Therefore, the suppression of the bacterial replication revealed inhibitory mechanism of β-caryophyllene against *H. pylori* growth. β-caryophyllene is found to be present in a numbers of plant materials (Table 1), indicating that the EO-bearing plants could suppress the expressions of replication genes in *H. pylori*, thereby inhibiting the bacterial growth.

Then, *H. pylori* uses its polar flagella (encoded by *flaA* and *flaB* genes) for motility (62) and encodes various *adhesion factors* such as adherence-associated lipoproteins (AlpA and AlpB) (Fig. 1) and blood group antigen-binding adhesion (BabA) (encoded respectively by the genes *alpA, alpB*, and *babA*) for successful binding to gastric epithelium cells (63, 64). Treatment of *H. pylori* with patchouli alcohol at 10 and 20 µg/mL considerably reduced the expression levels of *H. pylori* motility-related genes (flaA and flaB) (22). Patchouli alcohol at concentrations of at 5, 10, or 20 µg/mL could also significantly down regulate the expression levels of alpA and alpB, but had no significant effect on that of babA (22).

Finally, *H. pylori* releases two virulence factors such as vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA) (Fig. 1), causing host tissue damage after successful colonization (62). VacA causes multiple cellular effects, including cell vacuolation, mitochondrial stress and dysfunction, membrane potential depolarization, autophagy, inhibition of T cell activity, and apoptosis (65). CagA is strongly associated with the gastric mucosa-associated lymphoid tissue (MALT) lymphoma (66). The EO of *A. lancea*. at 1/2 × MIC was reported to markedly decrease the translocation levels of CagA protein in GES-1 cells at 4, 8, and 12 h of incubation, although *CagA* mRNA level in the cells was found to increase at the same treatment conditions (51). Phytoncide EO (containing α-pinene 61.56 mg/g) steam-distillated from pinecone of *P. koraiensis* Siebold & Zucc. was reported to have various pharmacological effects and its efficacy includes antioxidants, immune stimulation, anti-cancer, and anti-inflammatory activities (8). The phytoncide reduced gastric severity in *H. pylori*-infected mice via down regulation of the expression levels of *CagA* gene in the gastrointestinal system of C57BL/6 mice. In addition to the growth-inhibiting activity, β-caryophyllene suppressed the translocation of CagA proteins into *H. pylori*-infected AGS gastric cancer cells via down regulating transcription of type IV secretion system (T4SS) components (such as *virB2*, *virB4*, and *virB8*) involved in CagA injection into the host cell (45). β-Caryophyllene also decreased transcription of secretion system subunit protein A (SecA) of type V secretion system (T5SS) involved in VacA secretion (45) (Fig.1), thereby producing anti-inflammatory, anticancer, and antibacterial effects (44).

These studies have demonstrated the effect of plant EOs and mode of action of some selected EO components against cell morphology, membrane integrity, motility,

growth, and colonization capacity of *H. pylori*. However, the development of peptic ulcer diseases and gastric cancer is a long-term process specially affected by interaction of multiple factors such as gastric environmental, host genetic, and bacterial virulence factors. Therefore, further researches need to be carried out for better understanding of mechanisms related to effects of plant EOs and their components against the expression of the bacterial virulence factors and their role in the pathogenic mechanism.

In vivo effect on H. pylori

Gastric cancer is known as a highly lethal disease and *H. pylori* infection is one of the risk factors causing gastritis and gastric cancer. *In vitro* studies have revealed the potential antibacterial activity of plant EOs and their constituents against several multiple-drug resistant strains of *H. pylori*. However, their anti-*H. pylori* activity could be affected by the harsh acidic environment of the stomach. Therefore, *in vivo* studies were performed to demonstrate therapeutic effect of plant EOs against *H. pylori* infection in mouse models. Oral application of carrot seed oil (*D. carota*) to *H. pylori* infected mice did not cause significant decreases in the bacterial densities in the treated group compared with those in the control group even though the EO was shown to exert an *in vitro* antibactericidal activity against *H. pylori* (7). Similarly, the lemongrass EO (*C. citratus*) was reported to completely eradicate *H. pylori* from the gastric mucosa of only 10% of *H. pylori*-infected mice (14). However, the bacterial colonies recovered from the stomachs of mice treated with the EO were considerably decreased in comparison with those in the untreated group (14). *H. pylori*-infected mice treated with 0.3% of *P. lactiflora* EO showed no *H. pylori* infection in stomachs after 10 day oral treatment while only in three *H. pylori*-infected mice with a significant decrease in *H. pylori* colonies were found in the group of 15 mice treated with 0.2% of the EO (49). The density of *H. pylori* was also found to significantly decrease in the stomach of mice fed with the EO of *Cinnamomum osmophloeum* Kaneh. (67). Mixture of *S. hortensis* and *O. vulgare* subsp. *hirtum* EOs (at the rate of 2:1 v/v) had a high efficiency in *H. pylori* eradication with only 30% of the treated mice remained positive for *H. pylori* infection confirmed by the PCR analysis (68). The phytoncide EO of *P. koraiensis* exhibited a significantly inhibitory effect on the survival of *H. pylori* in the gastrointestinal system of C57BL/6 mice at 10 and 25 mg/mL. It also had a role in reducing gastric severity in *H. pylori*-infected mice via down regulation of the expression levels of pro-inflammatory cytokines in the gastric mucosa and the cytotoxin CagA gene (8).

Some EO components could efficiently alleviate the bacterial infection and colonization, and inhibit inflammation of gastric tissue in *H. pylori*-infected mice, thereby possessing antitumor activity. Effect of patchouli alcohol on *H. pylori* eradication was reported to effectively attenuate gastritis with less bacterial resistance and mitigate *H. pylori* colonization in C57BL/6 mice stomach (22). The results from the rapid urease test (RUT) showed that the *H. pylori* eradication rates were 100 and 80% in the treatments of triple therapy and patchouli alcohol (5 mg/kg), respectively, while the rate in both of the treatments was 40% using boracic acid methylene blue (BAMB) staining, suggesting that the *H. pylori* detection sensitivity of histologic section staining was higher than that of the RUT (22). Treatments of *H. pylori*- induced ulcer in rats with geraniol alone or in combination with clarithromycin revealed that geraniol had a significant anti-ulcer effect by attenuating inflammation, oxidative stress and *H. pylori* infection in the gastric mucosa (69) while the compound showed an *in vitro* bactericidal action against *H. pylori* with MBC of 100 µg/mL (7). Both the *in vivo* treatments produced a 50% reduction of *H. pylori* infection (69). Despite low anti-*H. pylori* activity shown in *in vitro* tests (Table 2), β-caryophyllene derived from steam distillate of dry flower buds of *Syzygium aromaticum* (L.) Merr. & L.M.Perry was shown to significantly eradicate *H. pylori* infection (56–80%) in infected male C57BL/6 mice with dose-dependent manner (100–500 mg/kg/day, once daily for 2 weeks) using the CLO (Campylobacter-like organism) test and real-time PCR (44). Orogastric treatments in *H. pylori*-infected *Mongolian gerbils* also showed that β-caryophyllene significantly reduced the degree of *H. pylori* infection after 6 and 12 weeks of treatments at both low doses (100 mg/kg) and high doses (500 mg/kg) (45). In addition to the *in vivo* bactericidal effect on *H. pylori*, β-caryophyllene improved inflammation of the gastric mucosa in mouse models (44, 45).

Widespread emergence of multidrug resistant strains of *H. pylori* and the inability of new antibiotics to eradicate their infections has drawn the attention of scientific community to explore the therapeutic benefits of plant EOs and their bioactive constituents. They could efficiently interfere in the infection process of *H. pylori* and mitigate gastritis with healing effect and minimal or no bacterial resistance.

Conclusion

EOs from different plant species and their constituents have been introduced as potent antimicrobial agents, especially against multidrug-resistant strains of *H. pylori,* in both *in vitro* and *in vivo* studies. In general, the results from the reviewed studies substantiate that plant EOs and active volatile components could act on multiple targets on the bacterial cell such as morphology, motility, expression of virulence genes and their protein levels. The natural preparations and volatile compounds could have potential growth-inhibiting activity and be helpful in attenuating the deleterious effects such as cytoplasmic vacuolation and apoptotic cell death induced by *H. pylori*.

Therefore, anti-*H. pylori* properties of plant essential oils, especially synergy among herbs, effective parts, and pure compounds warrant further studies to identify and completely decipher their modes of action. The positive effects of plant essential oils against *H. pylori* indicated that they could be used as a new antimicrobial agent or functional health food for preventing and controlling the bacterial infection.

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Authors contributions

All authors have contributed equally in composing the manuscript.

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