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Original research article

# Exploring the potential of *Cinnamomum zeylanicum* oil against drug resistant *Helicobacter pylori*-producing cytotoxic genes

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**Abstract**
Thirty-one of sixty dyspeptic patients tested positive for *Helicobacter pylori* colonization in this study, as determined by histopathology and 16S rRNA. The cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin A (*vacA*) genes were found in 67.7 and 93.5% of *H. pylori* patients, respectively. The *cagA* gene was found to be associated with 100% of patients with duodenal erosion and ulceration identified via endoscopy examination. In addition, 86.7% of patients with cancerous and precancerous lesions, glandular atrophy, and intestinal metaplasia identified *via* histopathology examination. The *vacA s1m1* mutation was associated with more severe forms of gastric erosion and ulceration, as well as the presence of precancerous and cancerous lesions. Eighteen (64.3%) of the twenty-eight isolates were classified as multi-drug resistant (MDR) or pan-drug resistant (PDR) *H. pylori*. Due to a resurgence of interest in alternative therapies derived from plants as a result of *H. pylori* resistance to the majority of commonly used antibiotics, the inhibitory activity of five essential oils extracted from some commonly used medicinal plants was evaluated *in vitro* against drug-resistant *H. pylori* clinical isolates. *Cinnamomum zeylanicum* essential oil demonstrated the highest anti-*H. pylori* activity when compared to the other essential oils tested. Cinnamaldehyde was the most abundant compound in *C. zeylanicum* (65.91%). The toxicological evaluation established the safety of *C. zeylanicum* oil for human use. As a result, *C. zeylanicum* essential oil may represent a novel antibacterial agent capable of combating drug-resistant *H. pylori* carrying cytotoxin genes.

**Keywords:** Antimicrobial agents; Cinnamaldehyde; *Cinnamomum zeylanicum*; Cytotoxin-associated genes; Drug-resistance; *Helicobacter pylori*

**Highlights:**
- Conventional antibiotics have limitations due to *H. pylori* drug-resistance.
- Cinnamon is demonstrated as an effective antibacterial agent.
- Cytotoxic genes are importantly associated with detection of *H. pylori*.

## Introduction

*Helicobacter pylori* is a Gram-negative, microaerophilic, curved rod bacterium with lophotrichous flagella that colonizes several stomach areas after penetrating the mucous layer (Graham and Dore, 2016; Shapla et al., 2018). *H. pylori* infection is typically acquired in childhood and, if left untreated, frequently persists into adulthood (Karkhah et al., 2019). Infection with *H. pylori* is recognized as a global health issue affecting more than half of the world's population. In developing countries, infection rates exceed 90% due to low socioeconomic status, overcrowded living conditions, and poor environmental personal hygiene. In comparison, infection prevalence rates in developed countries are low (15.5%) (Mabeku et al., 2018).

*H. pylori* infection is linked to a variety of gastrointestinal disorders, including gastric lymphoma, peptic ulcer disease, chronic gastritis, and gastric carcinoma, and these effects are linked to *H. pylori* cytotoxic factors, cytotoxin-associated gene A (*cagA*), and vacuolating cytotoxin A (*vacA*) (Suzuki et

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al., 2019; Yamaoka and Graham, 2014). Because most epidemiological studies have reported that the attributable risk of gastric carcinoma caused by *H. pylori* infection is approximately 75%, the World Health Organization (WHO) has classified *H. pylori* as a type 1 carcinogen (Fiorentino et al., 2013). Approximately 70% of *H. pylori* strains have the *cagA* gene and express the high molecular weight protein CagA, which is transported into peptic epithelial cells and exerts toxic effects such as induction of cellular hyperproliferation, apoptosis, and loss of cellular ability to maintain its typical cytoskeletal structure, leading to pre-neoplastic and then neoplastic lesions (Tegtmeyer et al., 2011).

The variable combination of the signal (*s*) and mid (*m*) regions of the *vacA* gene results in varying cytotoxic levels of *vacA*, implying that specific *vacA* genotypes are associated with more severe forms of inflammation, injury, and an increased risk of precancerous and cancerous pathology (Chauhan et al., 2019; Graham et al., 2009). VacA can cause membrane-channel pores, mitochondrial cytochrome c depletion followed by apoptosis, and cell-membrane receptor attachment leading to an inflammatory response, in addition to inducing vacuolation. Furthermore, it promotes the persistence of *H. pylori* infection and can increase bacterial resistance to antibiotics (Ansari and Yamaoka, 2020; Omar et al., 2014). Standard triple therapy for *H. pylori* infection consists of proton pump inhibitors such as omeprazole combined with two antibiotics, most commonly clarithromycin and amoxicillin or metronidazole (McNicholl et al., 2020). However, widespread antibiotic resistance is on the rise. High therapy costs, poor patient compliance, a lack of essential antibiotics in developing-country rural areas, and treatment side effects all contribute to an increase in multidrug resistance (MDR), a global problem that necessitates new eradication protocols with minimal or no side effects (Ali et al., 2016, 2017, 2019, 2020; El-Shouny et al., 2020; El-Zawawy and Ali, 2016).

The vast majority of the world's population relies solely on medicinal plants for natural medicines (Al-Tohamy et al., 2018; El-Shouny et al., 2020; Miranda, 2021). According to the WHO, approximately 80% of the world's population relies on herbal medicines as their primary source of health care (Wang and Yu, 2015). Essential oils are aromatic compounds found in various plant organs that contain a mixture of volatile and odorous substances that have been used in folk medicine since antiquity (Aleksic and Knezevic, 2014). Essential oils have long been recognized for their medicinal properties, including antibacterial, antifungal, anticancer, antioxidant, and anti-inflammatory properties (Bouyahya et al., 2020). In recent years, the anti-*H. pylori* activity of essential oils from medicinal plants has been studied as an alternative therapy for treating disorders caused by *H. pylori* infection (Korona-Glowniak et al., 2020). *Cinnamomum zeylanicum*, also known as cinnamon, is a member of the Lauraceae family and has long been used as a health-promoting agent with antimicrobial properties (Nabavi et al., 2015). Cinnamon has been shown to have anti-ulcer activity and to be effective at inhibiting the growth of *H. pylori* as a traditional remedy for stomach and intestinal problems (Hamidpour et al., 2015). Cinnamaldehyde, the active ingredient in cinnamon essential oil, has immune-regulating and anti-inflammatory properties (Li et al., 2020). Aside from that, cinnamon essential oil has been shown to protect against gastroenteritis (Azab et al., 2017). To the best of our knowledge, this is the first report on the biomedical potential of *C. zeylanicum* essential oil against cytotoxin-associated genes that produce drug-resistant *H. pylori*. The characterization and cytotoxicity activity of *C. zeylanicum* were investigated in order to assess the performance of this essential oil as a new leading structure in the biomedical and pharmaceutical applications.

## Materials and methods

### *Gastric biopsy specimen collection and histopathological examination*

Biopsy specimens were collected from 60 dyspeptic patients who complained of upper gastrointestinal symptoms between 2019 and 2020. The required biopsies in this study were obtained from the antral gastric mucosa of the studied patients using Olympus forceps (Olympus X Q40) at Gastrointestinal Endoscopy Unit of Tanta University Hospital, Tanta, Egypt. Each patient had three antral biopsies taken. The collected gastric biopsy specimens were prepared as shown in the experimental setup (Fig. 1). The first biopsy was transported in 10% neutral buffered formalin until it was processed, stained, and examined at Histopathology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. The second biopsy sample was taken from the gastric antrum and placed directly in tubes containing phosphate buffer saline (PBS). It was then transported to the Microbiology Department, Faculty of Medicine, Al-Azhar University, and stored at –80 °C until the PCR assay for detection of 16S rRNA genes and *cagA/vacA* genotypes. The third antral biopsy was directly placed in sterile tubes containing 1ml of sterile saline solution and transported to Microbiology Laboratory, Faculty of Science, Tanta University, for culturing. The histopathology samples were preserved and fixed in 10% neutral buffered formalin for 24 h before being processed and embedded in paraffin wax. H&E and Giemsa stains were used to stain slides made from each sample block. All slides were histopathologically examined for *H. pylori*, degree of inflammation, intestinal metaplasia, glandular atrophy, lymphocyte follicle formation, or malignancy, and the results were recorded (Wright and Kelly, 2006).

### *PCR assay*

Genomic DNA was extracted from biopsy specimens using a tissue genomic DNA Mini kit (Geneaid Biomed Ltd., Taiwan) in accordance with the manufacturer's instructions. After confirming the diagnosis of *H. pylori* with 16S rRNA gene detection using specific primers for each genotype, the *cagA* and *vacA* genotypes were detected by PCR in 31 gastric antral biopsies (Suppl. Table 1). The target genes were amplified using a thermal cycler (Biometra UNO Thermoblock, Analytik Jena, Botron, Germany), and then detected using 2% agarose gel electrophoresis in comparison to a 50 bp DNA ladder standard, as well as a negative control (without DNA). PCR assays were performed in a volume of 20 µl containing approximately 2 µg genomic DNA, 0.6 µl primer, and 4 µl 5× FIREPol (Solis BioDyne) ready to load master mix. Denaturation at 94 °C for 4 min was followed by 30 denaturation cycles at 94 °C for 1 min, annealing at 59 °C, 54 °C, and 52 °C for 16S rRNA, *cagA*, and *vacA*, respectively, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The amplicons for 16S rRNA, *cagA*, *vacA s1*, *vacA s2*, *vacA m1*, and *vacA m2* genes were 110, 400, 259, 286, 570, and 642 bp in length, respectively (Chisholm et al., 2001; Falsafi et al., 2009).

### *Biopsy specimen preparation*

Biopsy specimens were processed as soon as they were received (Fig. 1). Each mucosal tissue biopsy was ground individually in a tissue grinder until homogenate was formed. The processed biopsies were inoculated onto the surface of selective Colum-

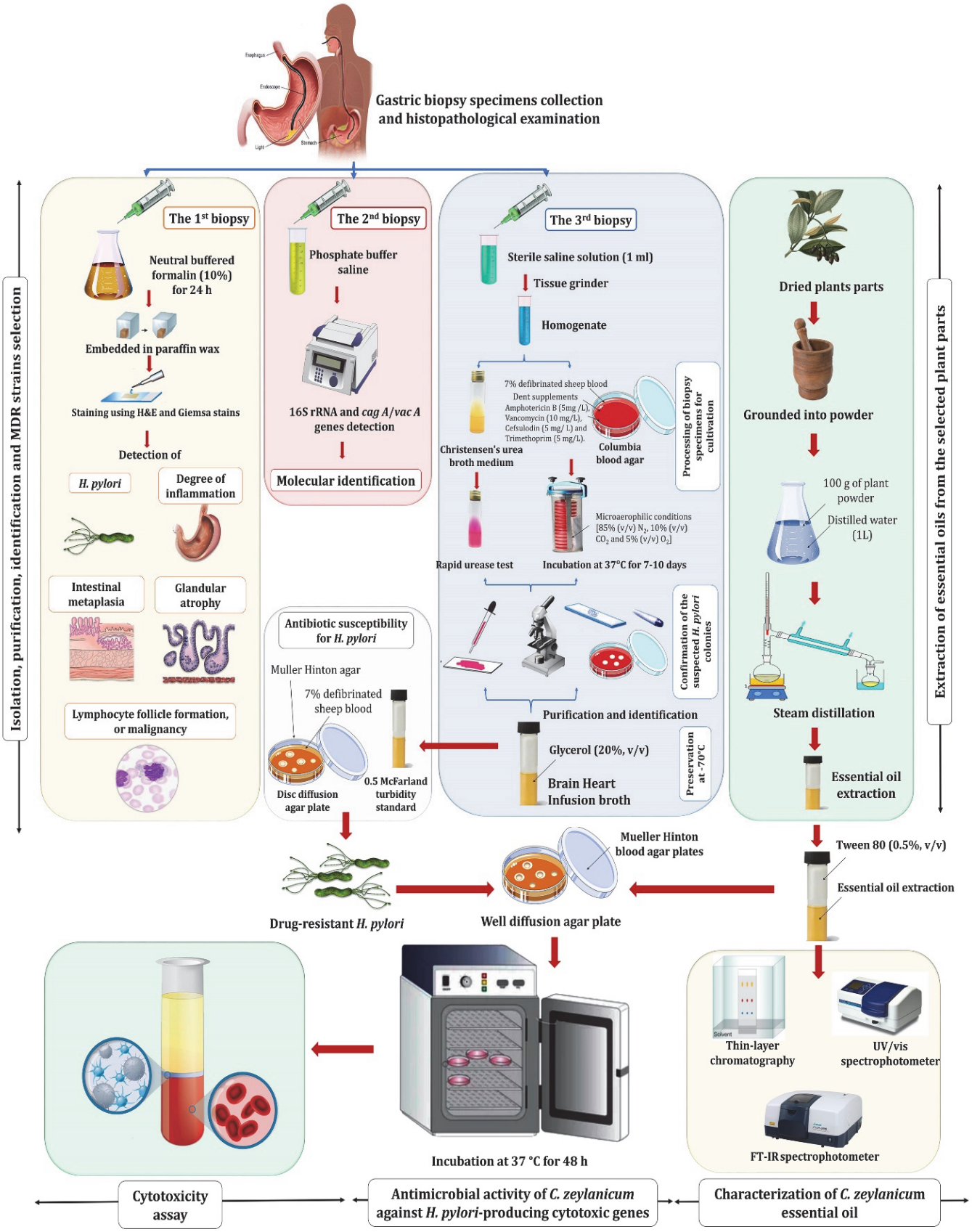


**Fig. 1.** Experimental set up for exploring the potential of *Cinnamomum zeylanicum* oil against drug resistant *Helicobacter pylori*-producing cytotoxic genes.

bia blood agar (Oxoid, Basingstoke, UK) plates containing 7% defibrinated sheep blood, and Dent supplements included amphotericin B (5 mg/l), vancomycin (10 mg/l), cefsulodin (5 mg/l), and trimethoprim (5 mg/l). Plates were incubated at 37 °C for 7–10 days in an anaerobic jar under microaerophilic conditions: $N_2$ (85%, v/v), $CO_2$ (10%, v/v), and $O_2$ (5%, v/v) were obtained using Campygen kits (Oxoid, UK) containing ascorbic acid as an active component. A portion of each patient's processed biopsy was placed directly into Christensen's urea broth medium and used for a rapid urease test to detect urease activity, which indicates the presence of *H. pylori* in the tested biopsy (Demiray-Gürbüz et al., 2017).

### *Confirmation of the suspected* H. pylori *colonies*

The plates were examined after incubation for alleged *H. pylori* colonies with the characteristic morphology. *H. pylori* was identified as a Gram-stained smear from typical colonies containing Gram-negative spiral-shaped microorganisms under light microscopy (Farinha and Gascoyne, 2005). The presence of *H. pylori* colonies on the selective plates was confirmed by urease, oxidase, and catalase positivity tests (Al Sulami et al., 2008). The clinical isolates of *H. pylori* were preserved in a small screw-capped tube in Brain Heart Infusion (BHI) broth medium (Oxoid, England) containing 20% glycerol (v/v) and stored at –70 °C until use.

### *Antibiotic susceptibility*

All *H. pylori* clinical isolates were tested *in vitro* using the disk diffusion method for their exposure to eight antibiotic discs (Oxoid, UK) of different classes, including metronidazole, clarithromycin, amoxicillin, tetracycline, Erythromycin, ciprofloxacin, levofloxacin, and amoxicillin / clavulanic acid (Fig. 1). A sterile cotton swab dipped in *H. pylori* suspension with a concentration equivalent to 0.5 McFarland turbidity standard was used to streak the surface of Muller Hinton (Oxoid, Basingstoke, UK) agar plates supplemented with 7% defibrinated sheep blood under aseptic conditions. Using sterile forceps, antibiotic discs were applied to the surface of the plates. Campygen kits were used to incubate the cultured plates for two days at 37 °C under microaerophilic conditions. The zone of inhibition (mm) was measured after the incubation period, and values were classified as sensitive or resistant using the Clinical and Laboratory Standards Institute (CLSI, 2012).

### *Antimicrobial activity of essential oils*

Five essential oils from various plants were chosen for this study based on a review of the literature and their importance in traditional medicine (Suppl. Table 2). According to Wong et al. (2014), a hundred grams of each plant part powder were covered with sufficient water in a distillation flask and subjected to steam distillation, which allows the simultaneous extraction of volatile oil where a colorless to a yellowish volatile constituent fraction with the characteristic odor was obtained, and the quantity was measured (Fig. 1). The essential oils were obtained and stored at 4 °C until further use. Oils were used in their crude form. To increase oil solubility in the media, 0.5% (v/v) Tween 80 was added to the oil. The inhibitory activity of essential oils against drug-resistant *H. pylori* isolates was screened using the agar well diffusion method (El-Shouny et al., 2020). A fixed volume (100 µl) of each essential oil was added separately into the wells of Mueller Hinton blood agar plates that had been cultivated with standardized *H. pylori* inoculum ($10^6$ CFU/ml). Under microaerophilic conditions, the plates were incubated for two days at 37 °C. Dimethyl sulphoxide was used as a negative control, and levofloxacin (5 µg) was used as a positive control. The mean inhibition zone diameters were measured in millimeters.

### *Cytotoxicity assessment*

The cytotoxicity test was used to determine the treatment concentration of the selected *C. zeylanicum* essential oil that does not have a toxic effect on peripheral blood mononuclear cells (PBMCs), chosen as standard cell modeling for this test. The cytotoxicity test was carried out following EL-Adawi et al. (2012) using serial dilutions of *C. zeylanicum* oil ranging from 50 to 1.5 v/v.

### *Characterization of* C. zeylanicum *essential oil*

The oil components were analyzed using Claus 580/560S gas chromatography-mass spectroscopy (GC-MS) following the method described by Adinew (2014). Thin-layer chromatography (TLC) was used to separate the active constituents of *C. zeylanicum* essential oil, particularly cinnamaldehyde using a silica gel G60 F254 plate (E. Merck, Germany). The mobile phase was a 93:7 v/v mixture of toluene and ethyl acetate. The plate was sprayed with vanillin sulfuric acid reagent, which contained 1% vanillic acid and 10% sulfuric acid in ethanol. Cinnamaldehyde was used as a standard and the separation method was performed following Shahverdi et al. (2007). Following separation, the $R_f$ (retention factor) value of cinnamaldehyde in *C. zeylanicum* essential oil was calculated and compared to a standard value. After scratching the cinnamaldehyde band, Fourier transform infrared spectroscopy (FTIR) analysis and mass spectroscopy were performed to detect all of the chemical functional groups present in cinnamaldehyde and determine the safety of the oil using an IR spectrophotometer Perkin-Elmer 1430. The measurements were made at infrared spectra ranging from 400 to 4000 $cm^{-1}$ (Kenawy et al., 2019). Using the Perkin Elmer Lambda 4B UV/vis spectrophotometer, UV analysis confirmed the purification of the selected cinnamon essential oil.

### *Statistical analysis*

The data collected were manipulated using the Statistical Package for Social Science (SPSS) version 22. The quantitative data were presented as mean, standard deviations, and ranges, whereas the qualitative data were presented as numbers and percentages. The data were analyzed using appropriate statistical methods such as the independent *t*-test, one-way analysis of variance (ANOVA), and the Chi-square test. A *P*-value of 0.05 or less was considered statistically significant.

## Results

This study included sixty dyspeptic patients with upper gastrointestinal symptoms; 38 were males and 22 were females; their ages ranged from 18 to 65 years; and 58% of them lived in rural areas. However, 41.7% of them lived in urban areas (Table 1). For the detection of *H. pylori*, histopathology was performed on all patient samples. The *H. pylori* specific 16S rRNA gene was detected in DNA extracted samples from biopsies of *H. pylori*-positive patients determined by histopathology, and the results were identical. Sixty patients were classified as *H. pylori*-positive (51.7%), while 48.3% were *H. pylori*-negative. Despite the fact that the majority of *H. pylori*-positive patients were males and lived in rural areas, there was no statistically significant relationship between gender ($P$ = 0.095) or residence ($P$ = 0.327) and *H. pylori*-positive patients. There was also no correlation between age and *H. pylori* detection ($P$ = 0.986).

Endoscopic examination of *H. pylori*-positive patients revealed that 58.1% had gastritis, 19.4% had normal gastric mucosa, and 12.9% had gastric ulceration (Table 1). In contrast, 58.1% of *H. pylori*-positive patients had normal duodenal mucosa, while 16.1, 9.7, 3.2, 12.9, and 6.5% had duodenitis, duodenal ulcers, gastric cancer, gastric ulceration, and gastric erosions, respectively (Table 1). The detection of gastric ulcers was significantly higher in the *H. pylori*-positive group when compared to the *H. pylori*-negative group ($P$ = 0.045). At the same time, the normal duodenal mucosa in the *H. pylori*-negative group was significantly higher ($P$ = 0.037). However, there was no statistically significant difference between the two groups in terms of other endoscopic examination parameters.

The *H. pylori*-positive group's histopathological findings revealed that no patient had normal gastric mucosa. However, 35.5, 29, and 12.9%, respectively, had mild, moderate, and severe gastritis. Gastric erosion, ulceration, cancer, intestinal metaplasia, glandular atrophy, and lymphocyte follicle formation were found in 6.5, 12.9, 3.2, 9.7, 48.4, and 19.4% of the *H. pylori*-positive group, respectively (Table 1 and Fig. 2). Positive *H. pylori* gastric biopsies revealed pathological changes in the antral gastric mucosa as well as the presence of *H. pylori* in the lumen of the gastric glands. Gastric glands with foci of goblet cell metaplasia, abundant plasma cells in the lamina propria, and focal intraepithelial neutrophils are depicted in Fig. 2A. *H. pylori* organism colonizing the luminal border of the gastric gland in antral-type mucosa (Fig. 2B). Fig. 2C shows a gastric mucosal ulcer with normal glandular maturation to the surface and a significant inflammatory cell infiltrate. As shown in Fig. 2D, a poorly differentiated gastric adenocarcinoma (mucinous carcinoma) beyond the lamina propria with single cells with abundant cytoplasm and one large (mucin-filled) vacuole. There was a statistically significant relationship between the presence of glandular atrophy and the presence of *H. pylori* ($P$ = 0.001). In this study, intestinal metaplasia and lymphocyte follicle formation were found only in *H. pylori*-positive patients' biopsies, with a statistically significant difference in lymphocyte follicle formation only ($P$ = 0.228 and $P$ = 0.013,

**Table 1.** Demographic features, endoscopic and histopathological findings of patients' groups as regard *H. pylori* detection

| Variables | | | *H. pylori* +ve No. = 31 (51.7%) | *H. pylori* –ve No. = 29 (48.3%) | Total No. = 60 (100%) | *P*-value |
|---|---|---|---|---|---|---|
| Demographic features | Age | Range | 19–65 | 18–63 | 18–65 | 0.986** |
| | | Mean | 39.81 ± 12.09 | 39.86 ± 12.54 | 39.83 ± 12.20 | |
| | Sex | Male | 21 (67.7%) | 17 (58.6%) | 38 (63.3%) | 0.095* |
| | | Female | 10 (32.3%) | 12 (41.4%) | 22 (36.7%) | |
| | Residence | Rural | 20 (64.5%) | 15 (51.7%) | 35 (58.3%) | 0.327* |
| | | Urban | 11 (35.5%) | 14 (48.3%) | 25 (41.7%) | |
| Endoscopic findings | Normal gastric mucosa | | 6 (19.4%) | 10 (34.5%) | 16 (26.7%) | 0.645* |
| | Gastritis | | 18 (58.1%) | 17 (58.6%) | 35 (58.3%) | 0.965* |
| | Gastric erosion | | 2 (6.5%) | 2 (6.9%) | 4 (6.7%) | 0.945* |
| | Gastric ulcer | | 4 (12.9%) | 0 (0%) | 4 (6.7%) | 0.045* |
| | Gastric cancer | | 1 (3.2%) | 0 (0%) | 1 (1.7%) | 0.380* |
| | Normal duodenal mucosa | | 18 (58.1%) | 24 (82.8%) | 42 (70%) | 0.037* |
| | Duodenitis | | 5 (16.1%) | 2 (6.9%) | 7 (11.7%) | 0.266* |
| | Duodenal erosion | | 5 (16.1%) | 2 (6.9%) | 7 (11.7%) | 0.266* |
| | Duodenal ulcer | | 3 (9.7%) | 1 (3.4%) | 4 (6.7%) | 0.334* |
| Histopathological findings | Normal gastric mucosa | | 0 (0%) | 1 (3.4%) | 1 (1.7%) | 0.297* |
| | Gastritis | Mild | 11 (35.5%) | 18 (62.1%) | 29 (48.3%) | 0.213* |
| | | Moderate | 9 (29%) | 6 (20.7%) | 15 (25%) | |
| | | Severe | 4 (12.9%) | 2 (6.9%) | 6 (10%) | |
| | Gastric erosion | | 2 (6.5%) | 2 (6.9%) | 4 (6.7%) | 0.945* |
| | Gastric ulcer | | 4 (12.9%) | 0 (0%) | 4 (6.7%) | 0.045* |
| | Gastric cancer | | 1 (3.2%) | 0 (0%) | 1 (1.7%) | 0.380* |
| | Intestinal metaplasia | Mild | 1 (3.2%) | 0 (0%) | 1 (1.7%) | 0.228* |
| | | Moderate | 2 (6.5%) | 0 (0%) | 2 (3.3%) | |
| | | Severe | 0 (0.0%) | 0 (0%) | 0 (0%) | |
| | Glandular atrophy | Mild | 10 (32.3%) | 0 (0%) | 10 (16.7%) | 0.001*9 |
| | | Moderate | 5 (16.1%) | 2 (6.9%) | 7 (11.7%) | |
| | | Severe | 0 (0.0%) | 0 (0.0%) | 0 (0%) | |
| | Lymphocyte follicles formation | | 6 (19.4%) | 0 (0.0%) | 6 (10%) | 0.013* |

* Chi-square test, $P$-value ≤ 0.05 is considered significant. ** Independent $t$-test, $P$-value ≤ 0.05 is considered significant.

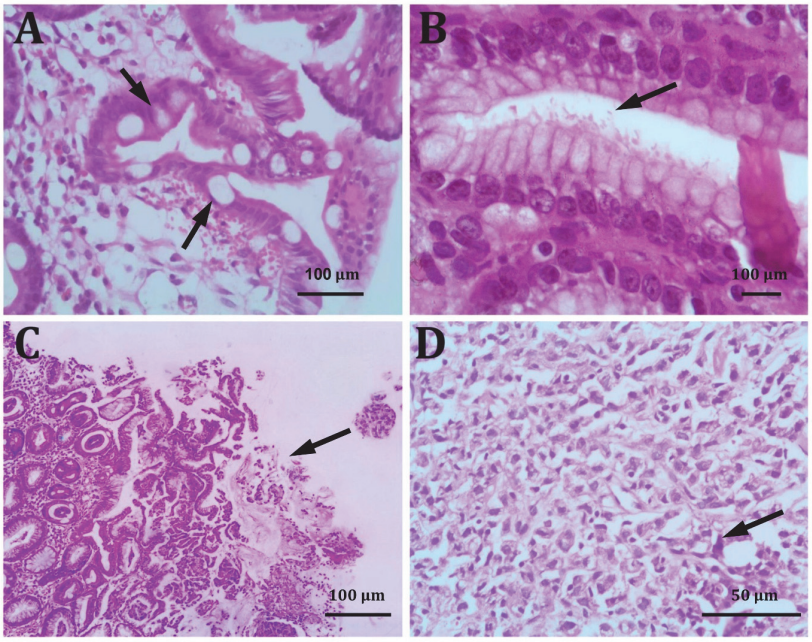


**Fig. 2.** Histopathological findings of the studied patients. Intestinal metaplasia: Gastric glands showing foci of goblet cell metaplasia and active inflammation (H, E, ×400) (**A**). Antral mucosa with *H. pylori* colonization (H, E, ×1000) showing the curved rods *H. pylori*, colonize the luminal border of the gastric gland (**B**). Gastric ulcer with inflammatory cell infiltration (H&E, ×50) (**C**). Gastric cancer: Mucinous adenocarcinoma (H&E, ×400) (**D**).

respectively). Along with the endoscopic findings, gastric ulcer detection was significantly higher in the *H. pylori*-positive group ($P$ = 0.045). The only case of gastric cancer ($P$ = 0.380) was found in the *H. pylori*-positive group.

In addition to the 16S rRNA confirmation of *H. pylori* (Suppl. Fig. 1), the cytotoxic genes *vacA* and *cagA* were also detected and studied (Tables 2, 3 and Suppl. Fig. 2). The *vacA* gene alone was found in eight biopsy specimens (25.8%). Only two specimens (6.5%) of the *H. pylori*-positive patients tested negative for the cytotoxic *cagA* and *vacA* genes. The *cagA* gene was found in 67.7% of *H. pylori*-positive patients. In addition, the *vacA* gene was found in 93.5% of *H. pylori*-positive patients. Furthermore, in samples from *H. pylori*-positive patients, the predominant *vacA* m allele was *m2* (58.1%), and the predominant *s* allele was *s1* (54.8%). The *vacA m1* and *s2* alleles were found in 35.5 and 38.7% of the cases, respectively. The most common genotype of the allelic combination of the *vacA* gene in this study was *s2m2* (38.7%), with *s1m1* and *s1m2* accounting for 35.5 and 19.4% of *H. pylori*-infected patients, respectively. Furthermore, no patient had the subtype *s2m1*.

The *cagA* gene was found to be positive in all eight cases of duodenal erosion and ulceration seen by endoscopy (Table 2). In addition, the *cagA* gene was found in 86.7% of cancerous and precancerous lesions, glandular atrophy, and intestinal metaplasia identified by histopathology (Table 3 and Fig. 3A). Endoscopy and histopathology revealed that *vacA s1* and *m1* were significantly associated with gastric erosion ($P$ = 0.013) and ulceration ($P$ = 0.006), respectively. Furthermore, precancerous and cancerous lesions detected by histopathology were significantly associated with *vacA s1* ($P$ = 0.045) and *m1* ($P$ = 0.001), respectively. These two alleles, however, were found not to be associated with normal gastric mucosa by endoscopy ($P$ = 0.003 and $P$ = 0.043, respectively) and mild gastritis by histopathology ($P$ = 0.002).

The *vacA s2* and *m2* alleles, on the other hand, were found to be significantly associated with normal gastric mucosa by endoscopy ($P$ = 0.001 and $P$ = 0.020, respectively) and mild gastritis by histopathology ($P$ = 0.004 and $P$ = 0.006, respectively). However, the latter two alleles were not associated with the more severe forms, including gastric erosion ($P$ = 0.030) and ulceration ($P$ = 0.022) found by endoscopy and histopathology, as well as the presence of precancerous ($P$ = 0.038) and cancerous ($P$ = 0.001) lesions. Furthermore, *vacA s1m1* was linked to the more severe gastric erosion by endoscopy and ulceration by histopathology ($P$ = 0.006), as well as the presence of precancerous and cancerous lesions ($P$ = 0.001). *VacA s2m2* on the other hand was linked to normal gastric mucosa by endoscopy ($P$ = 0.001) and mild gastritis by histopathology ($P$ = 0.004). Fig. 3B, C depicts the relationship between different *vacA* genotypes and gastric endoscopy and pathology. As a result, no association has been found between the *cagA* gene and any *vacA* genotype, demonstrating that the effect of *vacA* genotypes, particularly *s1m1* and alleles, is not a reflection of the *cagA* gene's impact.

In this study, 28 (46.6%) of 60 cultivated gastric antral biopsy samples were positive for *H. pylori* culture, and 90.1% of patients with *H. pylori* were positive by histopathology. Twenty-eight *H. pylori* clinical isolates were obtained from cultivated gastric biopsy specimens and identified using colony morphology, which showed small, transparent colonies on the surface of Columbia blood agar (Suppl. Fig. 3). *H. pylori* is Gram-negative, curved rod-shaped, and spiral-shaped, according to microscopic examination of a bacterial film stained with Gram stain (Suppl. Fig. 3). All positive *H. pylori* cultures were subjected to biochemical tests, and the results revealed that they were all oxidase, catalase, and urease positive (Suppl. Fig. 4). Additionally, gastric biopsy specimens used for the rapid urease test yielded positive results due to the rapid development of pink color (Suppl. Fig. 4).

**Table 2.** Frequency of Toxins genes by PCR and their relation to endoscopic findings in *H. pylori*-positive patients

| Toxins genes | | | Endoscopic findings | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Gastric findings No. (%) | | | | | | | | Duodenal findings No. (%) | | | | | |
| | | | Normal mucosa | | Gastritis | | Gastric erosion/ ulceration | | Gastric cancer | | Normal mucosa | | Duodenitis | | Duodenal erosion/ ulceration | |
| | | | 6 (19.4%) | *P** | 18 (58.1) | *P** | 6 (19.4) | *P** | 1 (3.2) | *P** | 18 (58.1%) | *P** | 5 (16.1) | *P** | 8 (25.8) | *P** |
| *cagA*-positive | | 21 (67.7%) | 4 (66.7%) | 0.950 | 11 (61.1) | 0.353 | 5 (83.3) | 0.363 | 1 (100) | 0.483 | 11 (61.1) | 0.353 | 2 (40) | 0.147 | 8 (100) | 0.023 |
| *vacA/cagA*-negative | | 2 (6.5%) | 0 (0) | 0.474 | 2 (11.1) | 0.214 | 0 (0) | 0.474 | 0 (0) | 0.790 | 2 (11.1) | 0.214 | 0 (0) | 0.521 | 0 (0) | 0.389 |
| *vacA*-positive | *s1m1* | 11 (35.5%) | 0 (0) | 0.043 | 5 (27.8) | 0.291 | 5 (83.3) | 0.006 | 1 (100) | 0.170 | 7 (38.9) | 0.981 | 2(40) | 0.818 | 4 (50) | 0.319 |
| | *s1m2* | 6 (19.4%) | 0 (0) | 0.181 | 5 (27.8) | 0.162 | 1 (16.7) | 0.853 | 0 (0) | 0.618 | 4 (22.2) | 0.634 | 2(40) | 0.202 | 0 (0) | 0.108 |
| | *s2m2* | 12 (38.7%) | 6 (100) | 0.001 | 6 (33.3) | 0.470 | 0 (0) | 0.030 | 0 (0) | 0.419 | 5 (27.8) | 0,291 | 1(20) | 0.348 | 4 (50) | 0.447 |
| *s1* | | 17 (54.8%) | 0 (0) | 0.003 | 10 (55.6) | 0.925 | 6 (100) | 0.013 | 1 (100) | 0.356 | 9 (50) | 0.524 | 4 (80) | 0.217 | 4 (50) | 0.750 |
| *m1* | | 11 (35.5%) | 0 (0) | 0.043 | 5 (27.8) | 0.291 | 5 (83.3) | 0.006 | 1 (100) | 0.170 | 5 (27.8) | 0.291 | 2 (40) | 0.818 | 4 (50) | 0.319 |
| *s2* | | 12 (38.7%) | 6 (100) | 0.001 | 6 (33.3) | 0.470 | 0 (0) | 0.030 | 0 (0) | 0.419 | 7 (38.9) | 0.981 | 1(20) | 0.348 | 4 (50) | 0.447 |
| *m2* | | 18 (58.1%) | 6 (100) | 0.020 | 11 (61.1) | 0.686 | 1 (16.7) | 0.022 | 0 (0) | 0.232 | 11 (61.1) | 0.686 | 3 (60) | 0.924 | 4 (50) | 0.592 |
| *vacA* only | | 8 (25.8%) | 2 (33.3) | 0.639 | 5 (27.8) | 0.768 | 1 (16.7) | 0.569 | 0 (0) | 0.549 | 5 (27.8) | 0.768 | 3 (60) | 0.056 | 0 (0) | 0.053 |

* Chi-square test, *P*-value ≤ 0.05 is considered significant.

**Table 3.** Toxins genes by PCR in relation to histopathology and *cagA* gene in *H. pylori*-positive patients

| Toxins genes | | | Histopathology and *cagA* gene | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Lesions by histopathology No. (%) | | | | | | | | | | *cagA* No. (%) | |
| | | | Mild gastritis | | Moderate/ severe gastritis | | Gastric erosion/ ulceration | | Precancerous and cancerous | | Lymphocyte follicles formation | | *cagA* 21 (67.7) | |
| | | | 11 (35.5%) | *P** | 13 (41.9) | *P** | 6 ( 19.4) | *P** | 15 (48.4) | *P** | 6 (19.4) | *P** | 21 (67.7) | *P** |
| *cagA*-positive | | 21 (67.7%) | 7 (63.6) | 0.717 | 8 (61.5) | 0.53 | 5 (83.3) | 0.363 | 13 (86.7) | 0.029 | 6 (100) | 0.060 | – | – |
| *vacA/cagA*-negative | | 2 (6.5%) | 1 (9.1) | 0.657 | 1 (7.7) | 0.811 | 0 (0) | 0.474 | 1 (6.7) | 0.962 | 0 (0) | 0.474 | – | – |
| *vacA*-positive | *s1m1* | 11 (35.5%) | 0 (0) | 0.002 | 5 (38.5) | 0.768 | 5 (83.3) | 0.006 | 10 (66.7) | <0.001 | 4 (66.7) | 0.075 | 9 (42.9) | 0.214 |
| | *s1m2* | 6 (19.4%) | 2 (18.2) | 0.902 | 3 (23.1) | 0.656 | 1 (16.7) | 0.853 | 1 (6.7) | 0.083 | 0 (0) | 0.181 | 4 (19) | 0.95 |
| | *s2m2* | 12 (38.7%) | 8 (72.7) | 0.004 | 4 (30.8) | 0.44 | 0 (0) | 0.030 | 3 (20) | 0.038 | 2 (33.3) | 0.763 | 8 (38.1) | 0.919 |
| *s1* | | 17 (54.8%) | 2 (18.2) | 0.002 | 8 (61.5) | 0.524 | 6 (100) | 0.013 | 11 (73.3) | 0.045 | 4 (66.7) | 0.517 | 13 (61.9) | 0.252 |
| *m1* | | 11 (35.5%) | 0 (0) | 0.002 | 5 (38.5) | 0.768 | 5 (83.3) | 0.006 | 10 (66.7) | <0.001 | 4 (66.7) | 0.075 | 9 (42.9) | 0.214 |
| *s2* | | 12 (38.7%) | 8 (72.7) | 0.004 | 4 (30.8) | 0.44 | 0 (0) | 0.030 | 3 (20) | 0.038 | 2 (33.3) | 0.763 | 8 (38.1) | 0.919 |
| *m2* | | 18 (58.1%) | 10 (90.9) | 0.006 | 7 (53.8) | 0.686 | 1 (16.7) | 0.022 | 4 (26.7) | 0.001 | 2 (33.3) | 0.172 | 12 (57.1) | 0.880 |
| *vacA* only | | 8 (25.8%) | 3 (27.3) | 0.89 | 4 (30.8) | 0.592 | 1 (16.7) | 0.569 | 1 (6.7) | 0.018 | 0 (0) | 0.108 | – | – |

* Chi-square test, *P*-value ≤ 0.05 is considered significant.

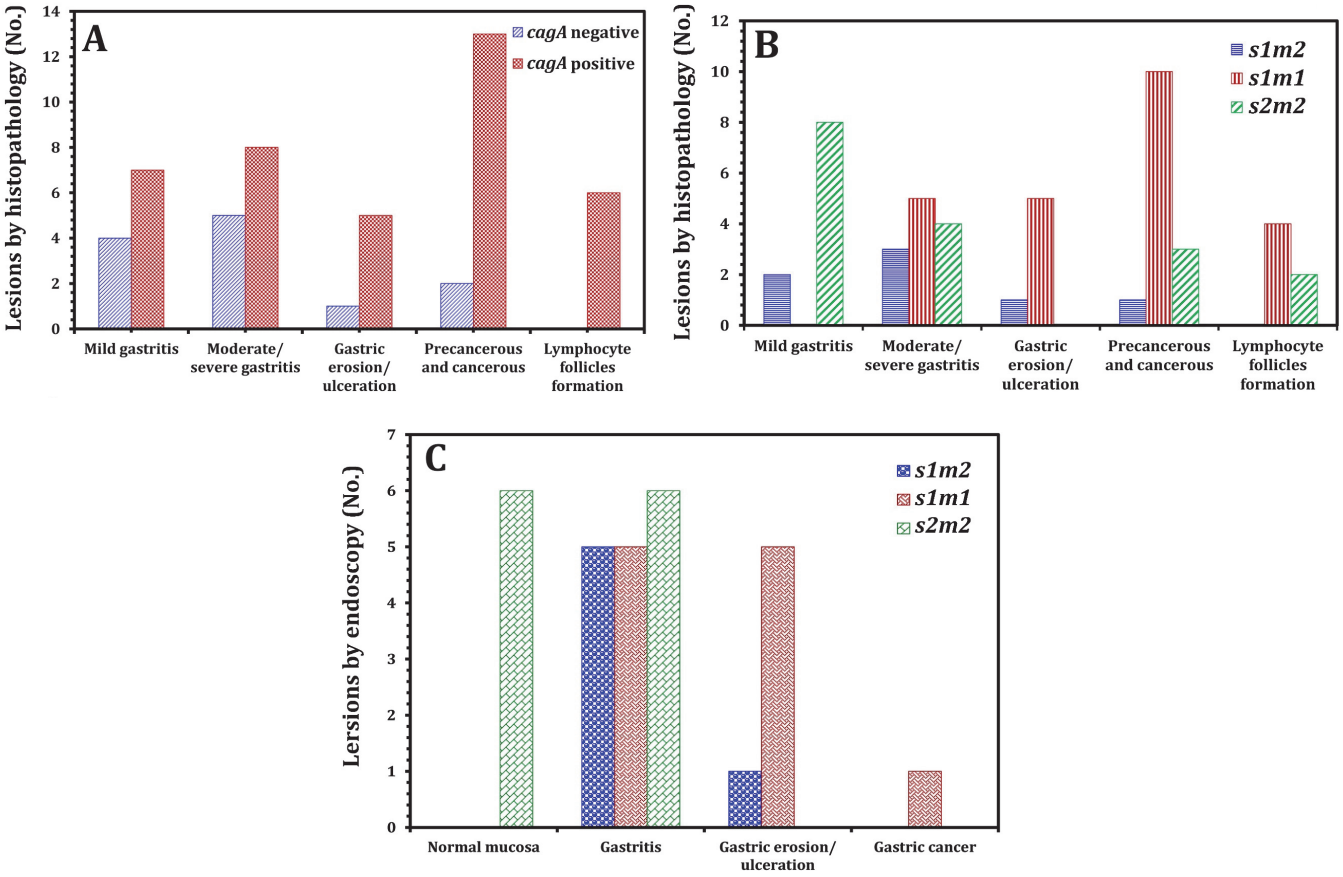


**Fig. 3.** The association between gastric pathology and *cagA* gene (**A**), different *vacA* genotypes (**B**), and different *vacA* genotypes with gastric endoscopy (**C**).

The cluster dendrogram results revealed multiple acquired antibiotic resistance among isolated clinical isolates (Fig. 4), with 18 out of 28 tested *H. pylori* proving to be drug resistant isolates, with six isolates (green color cluster) recording 100% resistance to all tested antibiotics (pan-drug resistant; PDR) and 12 isolates (blue color cluster) recording resistance to at least one antibiotic in three or more antibiotic classes and identified as MDR. The remaining 10 *H. pylori* isolates (pink color cluster) were sensitive to almost all antibiotics tested, with resistance rates of less than 50%. Metronidazole had the highest resistance (100%) of all 28 *H. pylori* clinical isolates, followed by amoxicillin and clarithromycin (78.6%) and erythromycin (57.2%), while levofloxacin (28.6%) had the lowest resistance, followed by tetracycline (39.3%) as depicted in Suppl. Table 3.

The demographics of the patients with drug-resistant *H. pylori* revealed that 72.2% were males and 27.8% were females, their ages ranged from 19 to 65 years, and 66.7% lived in rural areas. However, 33.3% of them were urban areas. Endoscopic examination of patients with drug-resistant *H. pylori* revealed that 61.1% had gastritis, 22.2% had gastric erosions/ulceration, 5.5% had gastric cancer, 16.7% had duodenitis, and 22.2% had duodenal erosions. According to histopathology, 22.2, 50, 22.2, and 61.1% of patients with drug-resistant *H. pylori* had mild gastritis, moderate/severe gastritis, gastric erosions/ulceration, and precancerous/cancerous lesions, respectively (Table 4). There were no statistically significant differences in demographic features, endoscopic and histopathological findings between patients with drug-resistant and sensitive *H. pylori* isolates. Furthermore, the *cagA* and *vacA* genes were found in 83.3 and 100% of patients with drug resistant *H. pylori*, respectively, with a higher frequency than in patients with sensitive *H. pylori*; however, this did not reach a statistically significant level.

The anti-*H. pylori* activity of five essential oils from different plants was investigated *in vitro* against 18 drug-resistant *H. pylori* clinical isolates using the agar well diffusion method (Fig. 5A). The results showed that all of the tested essential oils had inhibitory activity against drug-resistant *H. pylori* isolates with an inhibition zone diameter range of 0.0 and 29 mm, with *C. zeylanicum* oil having the most robust anti-*H. pylori* activity compared to the other oils, with a mean inhibition zone of 23.4 mm, which was higher than the levofloxacin positive control (16.7 mm), followed by *Syzygium aromaticum* (19.8 mm), *Thymus vulgaris* (15.5 mm), and *Rosmarinus officinalis* (9.8 mm) (Table 5 and Fig. 5B).

The cytotoxicity test results revealed that using *C. zeylanicum* oil was extremely safe on the tested PBMCs, with no $IC_{50}$ (the inhibitory concentration required to inhibit 50% of cell growth) even at the highest concentrations (Fig. 5C). As the concentration of *C. zeylanicum* oil was increased, the cell viability percentage decreased (inhibition rate increased) until it reached its maximum concentration, at which point the cell viability percentage was 54.1%.

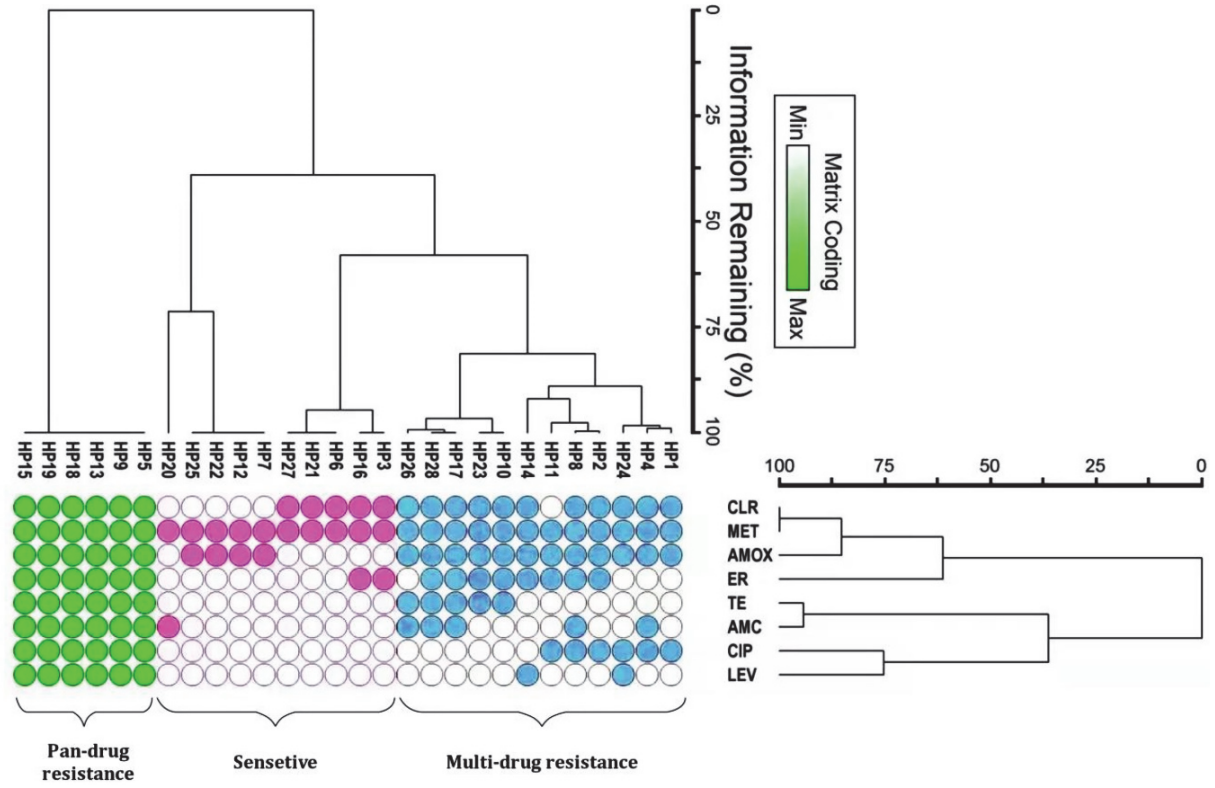


**Fig. 4.** Dendrogram profile of 28 *H. pylori* isolates (HP1–HP28). Blue color cluster (HP1, HP2, HP4, HP8, HP10, HP11, HP14, HP17, HP23, HP24, HP26, and HP28) represents MDR isolates. Green color cluster (HP5, HP9, HP13, HP15, HP18, and HP19) represents PDR isolates, while pink color cluster represents sensitive isolates. MTZ, metronidazole; LEV, levofloxacin; CLR, clarithromycin; CIP, ciprofloxacin; AMC, amoxicillin/clavulanic acid; ERY, erythromycin; AMOX, amoxicillin and TE, tetracycline.

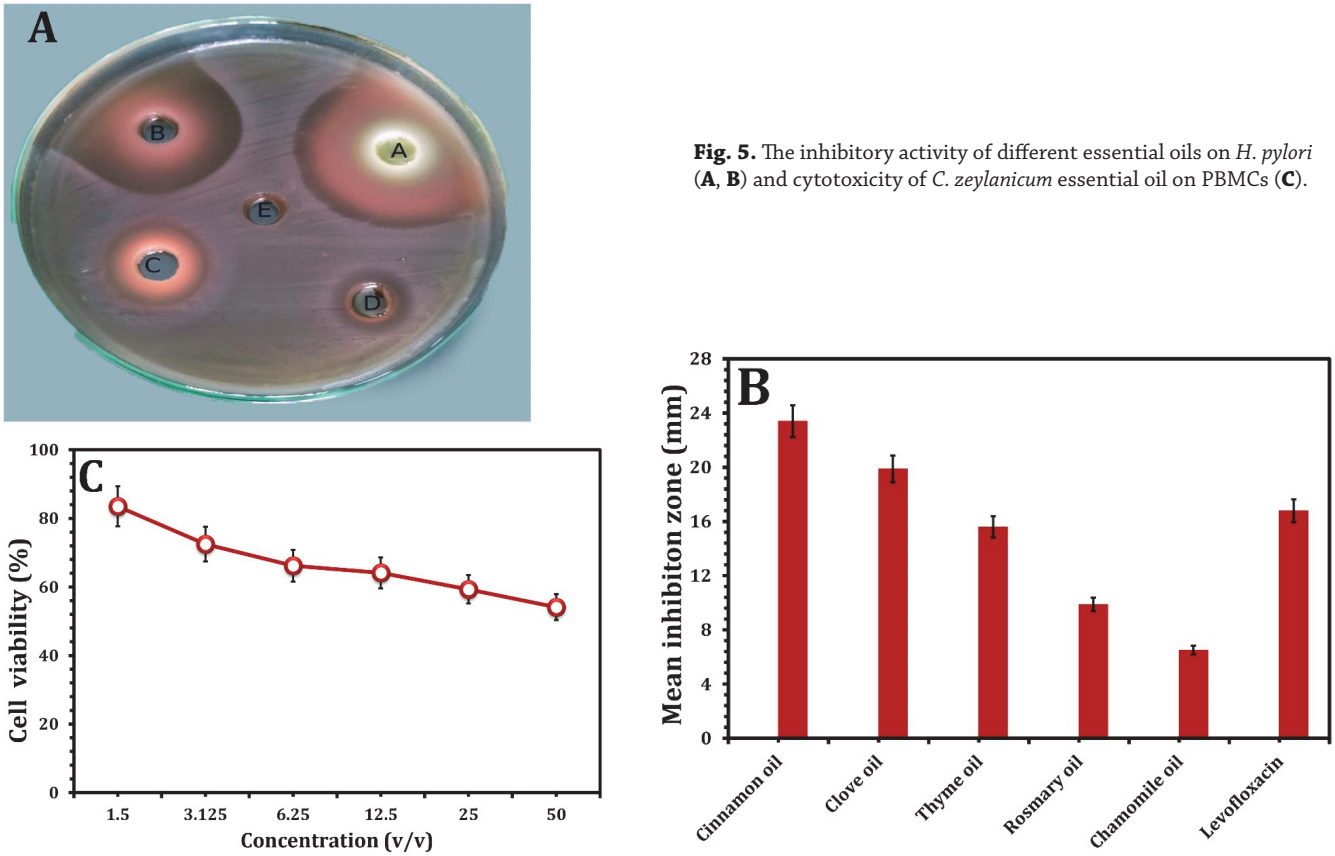


**Fig. 5.** The inhibitory activity of different essential oils on *H. pylori* (**A**, **B**) and cytotoxicity of *C. zeylanicum* essential oil on PBMCs (**C**).

**Table 4.** Comparison of patients with drug-resistant and drug-sensitive *H. pylori* isolates in terms of demographics, endoscopic and histopathological findings, and virulence genes

| Variables | | | Sensitivity results of *H. pylori* isolates *N* = 28 | | | | |
|---|---|---|---|---|---|---|---|
| | | | Drug resistant *H. pylori* | | Drug sensitive *H. pylori* | | *P*-value |
| | | | *N* = 18 | % = 64.3% | *N* = 10 | % = 35.7% | |
| Demographic features | Age | mean | 37.5 ± 11.7 | | 44.4 ± 10.64 | | 0.136** |
| | | range | 19-65 | | 32-62 | | |
| | Sex | Male (19) | 13 | 72.2% | 6 | 60% | 0.507* |
| | | Female (9) | 5 | 27.8% | 4 | 40% | |
| | Residence | Urban (10) | 6 | 33.3% | 4 | 40% | 0.724* |
| | | Rural (18) | 12 | 66.7% | 6 | 60% | |
| Endoscopic findings | Normal gastric mucosa (4) | | 2 | 11.1% | 2 | 20% | 0.520* |
| | Gastritis (17) | | 11 | 61.1% | 6 | 60% | 0.954* |
| | Gastric erosion / ulceration (6) | | 4 | 22.2% | 2 | 20% | 0.891* |
| | Gastric cancer (1) | | 1 | 5.5% | 0 | 0% | 0.448* |
| | Normal duodenal mucosa (15) | | 11 | 61.1% | 4 | 40% | 0.283* |
| | Duodenitis (5) | | 3 | 16.7% | 2 | 20% | 0.825* |
| | Duodenal erosion / ulceration (8) | | 4 | 22.2% | 4 | 40% | 0.592* |
| Histopathological findings | Mild gastritis(8) | | 4 | 22.2% | 4 | 40% | 0.318* |
| | Moderate to severe gastritis (13) | | 9 | 50% | 4 | 40% | 0.611* |
| | Gastric erosion / ulceration (6) | | 4 | 22.2% | 2 | 20% | 0.891* |
| | Precancerous and cancerous lesions (14) | | 11 | 61.1% | 3 | 30% | 0.115* |
| | Lymphocyte follicles formation (6) | | 4 | 22.2% | 2 | 20% | 0.891* |
| Toxic genes | *cagA* (20) | | 15 | 83.3% | 5 | 50% | 0.061* |
| | *vacA* (27) | | 18 | 100% | 9 | 90% | 0.172* |
| | *vacA* genotypes | *s1m1* (11) | 8 | 44.4% | 3 | 30% | 0.453* |
| | | *s1m2* (6) | 4 | 22.2% | 2 | 20% | 0.891* |
| | | *s2m2* (10) | 6 | 33.3% | 4 | 40% | 0.724* |
| | *vacA* alleles | *s1* (17) | 12 | 66.7% | 5 | 50% | 0.387* |
| | | *m1* (11) | 8 | 44.4% | 3 | 30% | 0.453* |
| | | *s2* (10) | 6 | 33.3% | 4 | 40% | 0.724* |
| | | *m2* (16) | 10 | 55.6% | 6 | 60% | 0.820* |

** Independent *t*-test, *P*-value ≤ 0.05 is considered significant. * Chi-square test, *P*-value ≤ 0.05 is considered significant.

Many components of the tested *C. zeylanicum* essential oil were confirmed by the GC-MS chromatograms (Table 6 and Suppl. Fig. 5). Cinnamaldehyde (65.91%) was the most abundant component, followed by coumarin (3.63%), caryophyllene (2.18%), eugenol (0.51%), trans-cinnamaylacetate (5.19%), cubenol (1.04%), alfa-terpineol (0.68%), alfa-copaene (8.33%), naphthalene (1.15%) and 2-propenal (0.43%). Cinnamaldehyde was identified as the primary element in *C. zeylanicum* oil by mass spectrometry, with a molecular ion peak at *m/z* 131 (Suppl. Fig. 5). The UV spectrum analysis revealed that the maximum absorption of *C. zeylanicum* essential oil is at 333 nm (Suppl. Fig. 5). The presence of various functional groups was revealed by the FTIR spectrum of *C. zeylanicum* oil (Suppl. Fig. 5). The peak at 1746 $cm^{-1}$ was assigned to stretching vibration of the carbonyl group of aldehyde (C = O), and the peak at 3009 $cm^{-1}$ was assigned to aromatic (C–H) stretching. The peaks at 2926 and 2855 $cm^{-1}$ were attributed to stretching alkanes (C–H), while the peaks at 1460 and 1684 $cm^{-1}$ were attributed to the presence of bending aromatic (C = C). The peak at 1163 $cm^{-1}$ corresponds to the stretching vibration of C–O, and the peak at 722 $cm^{-1}$ corresponds to the vibrational absorption (= CH) of benzene rings. These distinct peaks indicated that the tested *C. zeylanicum* essential oil contains a high concentration of aromatic and phenolic compounds, particularly cinnamaldehyde. The active compounds of the essential oil *C. zeylanicum* were isolated and the results showed that different spots had different $R_f$ values, with the primary active component having an $R_f$ of 0.634, which was very close to the $R_f$ value of standard cinnamaldehyde (Suppl. Fig. 6).

**Table 5.** Anti-*H. pylori* activity of essential oils of different plants in comparison to levofloxacin

| *H. pylori* codes | Essential oils | | | | | |
|---|---|---|---|---|---|---|
| | Zone of inhibition (mm) | | | | | |
| | Cinnamon oil | Clove oil | Thyme oil | Rosemary oil | Chamomile oil | Levofloxacin |
| 1 | 27.0 ± 0.0 | 22.1 ± 0.05 | 19.0 ± 0.0 | 17.2 ± 0.23 | 14.0 ± 0.0 | 32.0 ± 0.0 |
| 2 | 25.1 ± 0.1 | 19.5 ± 0.1 | 19.2 ± 0.25 | 12.0 ± 0.0 | 12.4 ± 0.17 | 27.6 ± 1.15 |
| 4 | 29.06 ± 0.05 | 24.3 ± 0.05 | 21.1± 0.17 | 17.0 ± 0.0 | 19.3 ± 0.21 | 25.0 ± 0.0 |
| 5 | 24.0 ± 0.0 | 19.0 ± 0.0 | 15.0 ± 0.0 | 15.2 ± 0.11 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| 8 | 26.1 ± 0.05 | 21.0 ± 0.0 | 17.7 ± 0.2 | 0.0 ± 0.0 | 0.0 ± 0.0 | 23.0 ± 0.0 |
| 9 | 22.0 ± 0.0 | 20.2 ± 0.1 | 20.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.00 |
| 10 | 25.0 ± 0.0 | 22.4 ± 0.11 | 18.1 ± 0.11 | 11.0 ± 0.0 | 15.0 ± 0.0 | 25.0 ± 0.0 |
| 11 | 20.06 ± 0.11 | 23.0 ± 0.0 | 21.0 ± 0.0 | 15.0 ± 0.0 | 13.1 ± 0.15 | 22.3 ± 0.5 |
| 13 | 23.0 ± 0.0 | 20.0 ± 0.0 | 21.06 ± 0.15 | 18.13 ± 0.11 | 0.0 ± 0.0 | 0.0 ± 0.00 |
| 14 | 19.13 ± 0.05 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 21.0 ± 0.0 |
| 15 | 25.0 ± 0.0 | 22.2 ± 0.15 | 18.9 ± 0.15 | 12.5 ± 0.0 | 13.0 ± 0.0 | 0.0 ± 0.00 |
| 17 | 20.3 ± 0.11 | 23.5 ± 0.05 | 17.5 ± 0.0 | 17.0 ± 0.0 | 16.3 ± 0.11 | 24.0 ± 0.0 |
| 18 | 17.0 ± 0.0 | 15.1 ± 0. 1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.00 |
| 19 | 23.0 ± 0.0 | 21.2 ± 0.1 | 16.0 ± 0.0 | 11.3 ± 0.15 | 0.0 ± 0.0 | 0.0 ± 0.00 |
| 23 | 20.2 ± 0.05 | 20.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 22.0 ± 0.0 |
| 24 | 28.0 ± 0.0 | 25.0 ± 0.0 | 22.03 ± 0.25 | 18.23 ± 0.25 | 15.0 ± 0.0 | 30.0 ± 0.0 |
| 26 | 22.2 ± 0.25 | 21.0 ± 0.05 | 18.0 ± 0.0 | 13.0 ± 0.0 | 0.0 ± 0.0 | 23.3 ± 0.5 |
| 28 | 25.1 ± 0.1 | 18.4 ± 0.15 | 16.03 ± 0.21 | 0.0 ± 0.0 | 0.0 ± 0.0 | 27.0 ± 0.0 |
| MIZ | 23.40 | 19.88 | 15.59 | 9.87 | 6.56 | 16.79 |
| *P*-value | 0.001*** | 0.000*** | 0.000*** | 0.000*** | 0.000*** | 0.000*** |

*P*-value ≤ 0.05 is considered significant. MIZ; Mean inhibition zone.

**Table 6.** GC-MS analysis of *C. zeylanicum* essential oil

| Retention time (min) | Peak area (%) | Name of compound | Molecular formula |
|---|---|---|---|
| 7.89 | 65.91 | Cinnamaldehyde | $C_9H_8O$ |
| 13.47 | 3.63 | Coumarin | $C_9H_6O_2$ |
| 15.19 | 2.18 | Caryophyllene | $C_{15}H_{24}$ |
| 16.73 | 0.51 | Eugenol | $C_{10}H_{12}O_2$ |
| 17.16 | 5.19 | Trans-cinnamaylacetate | $C_{11}H_{12}O_2$ |
| 17.76 | 1.04 | Cubenol | $C_{15}H_{26}O$ |
| 18.00 | 0. 68 | Alfa-terpineol | $C_{10}H_{18}O$ |
| 19. 15 | 8. 33 | Alfa-copaene | $C_{15}H_{24}$ |
| 19.87 | 1.15 | Naphthalene | $C_{10}H_8$ |
| 20.24 | 0.43 | 2-Propenal | $C_3H_4O$ |

## Discussion

Gastric biopsy histopathology and 16S rRNA testing are considered standard methods for detecting *H. pylori* infection (Tonkic et al., 2018). Histopathology detects not only *H. pylori* infection, but also the degree of inflammation and other pathologies such as gastritis, glandular atrophy, intestinal metaplasia, gastric cancer, or mucosa-associated lymphoid tissue lymphoma (Wang et al., 2015). The formation of lymphocyte follicles is a hallmark histopathological feature of *H. pylori*-induced gastric inflammation (Chen et al., 2002). The formation of lymphoid follicles within *H. pylori*-infected gastric mucosa is considered a mucous-associated lymphoid tissue that may be potential primary lesions for mucosa-associated lymphoid tissue lymphoma (Bashiri et al., 2019).

The *cagA* gene, which encodes the CagA protein and is found in nearly 60%–70% of *H. pylori* strains, is an essential cytotoxic gene (Fan et al., 2018). *VacA*, the other cytotoxic gene, encodes a vacuolating toxin excreted by *H. pylori* and is found in the majority of *H. pylori* strains (Ghotaslou et al., 2013b). The *vacA* gene has two variable regions: the (*s*) signal region (alleles *s1* and *s2*) and the (*m*) middle region (alleles *m1* and *m2*) (Miehlke et al., 2000). Zhang et al. (2008) found that the predominant *vacA m* allele was *m2*, and the predominant *s* allele was *s1*. In this study, the most common genotype of the *vacA* gene was *s2m2* (38.7%), while *s1m1* and *s1m2* were 35.5 and 19.4% of *H. pylori*-infected patients, respectively. These findings are consistent with previous findings (El-Shenawy et al., 2017; Boukhris et al., 2013). The subtype *s2m1* was not found in any of the patients in this study, which could be because the *vacA* genotype *s2m1* is uncommon (Matsunari et al., 2016). The variation in *vacA* genotypes and *cagA* gene frequencies in *H. pylori* isolates from various studies from various areas reflects the diverse nature of the *H. pylori* genome. It has been proposed that the genetic diversity of the *H. pylori* genome is greater than that of most other bacteria related to humans, and that the different patterns of genetic make-up of *H. pylori* strains are changeable, resulting in a geographic

distribution of *H. pylori* genotypes (van Doorn et al., 1998). Furthermore, the variability of *H. pylori* genes was used with modern technology to establish distinct phylogeographic parameters that allow even tracing of human migrations around the world (Ailloud et al., 2019).

Virulent *H. pylori* induce solid gastric inflammation, and the resulting gastric mucosal damage has been explained by bacterial cytotoxic factors expressed by cytotoxic genes (Rahimian et al., 2014). More severe forms of peptic pathology, such as peptic ulcer and gastric cancer, are commonly associated with the *cagA* gene, which is a marker for the presence of the *cag* pathogenicity island (*cag* PAI), which carries information for CagA secretion into human epithelial cells, with subsequent disruption of cell junctions and cellular damage; thus, *cagA*-positive *H. pylori* strains are more virulent than others (Higashi et al., 2005). *H. pylori* are more likely to develop glandular atrophy and intestinal metaplasia, owing to *cagA*-induced increased cell injury, which leads to impaired or abnormal repair of gastric glands (Testerman and Morris, 2014). Higher levels of *cagA* phosphorylation result in more cytoskeletal and mitogenic changes, which are more likely to be associated with gastric cancer (Argent et al., 2004).

Numerous studies have revealed that the prevalence of *H. pylori* antibiotic resistance varies significantly between countries (Gehlot et al., 2016). This could be attributed to geographical differences as well as the methodology used for an antibiotic sensitivity test. *H. pylori* antimicrobial resistance is a serious problem in developing countries, where resistance rates are higher than in developed countries (Jaka et al., 2018). In general, resistance to clarithromycin and metronidazole among *H. pylori* strains is increasing globally and varies across countries, according to *in vitro* studies (Ghotaslou et al., 2015). Fluoroquinolone resistance, on the other hand, is extremely low (less than 10%) globally (Fathi et al., 2013). Because antibiotic resistance is a major cause of therapy failure and can change over time, each country's monitoring of *H. pylori* antibiotic resistance, particularly for amoxicillin, clarithromycin, and metronidazole, should be warranted. In terms of demographics, endoscopic and histopathological findings, there are no significant differences between our patients with drug resistant and sensitive *H. pylori* isolates. *CagA* and *vacA* genes were found in 83.3 and 100% of patients with drug resistant *H. pylori* isolates, respectively, which was higher than in patients with sensitive *H. pylori* isolates. El-Shouny et al. (2020) found that 75% of PDR *H. pylori* strains possessed the *cagA* gene, while 87.5% possessed the *vacA* gene. Korona-Glowniak et al. (2019) reported that *cagA* and *vacAs1* genotypes were present in 75 and 62.5% of MDR *H. pylori* strains, respectively. Ghotaslou et al. (2013a) found no link between antibiotic resistance and the *cagA* gene. Except for metronidazole, which had a significant relationship with the *cagA* gene, Bachir et al. (2018) found no association between *vacA* or *cagA* genotypes and drug resistance. There was no link discovered between *H. pylori* resistance to clarithromycin or fluoroquinolones and the presence of specific virulence genes (Oktem-Okullu et al., 2020).

Among five essential oils derived from various plants, *C. zeylanicum* essential oil demonstrated the most potent anti-*H. pylori* activity. Cinnamon preparations have long been used to suppress *H. pylori* and treat gastrointestinal disorders with no side effects. These preparations have a high anti-*H. pylori* activity, which solves the problem of antibiotic resistance (Hamidpour et al., 2015). Korona-Glowniak et al. (2020), on the other hand, found that *C. zeylanicum* essential oil had potent activity against the reference *H. pylori* strain when they examined the inhibitory effect of 26 different essential oils against *H. pylori* ATCC strain *in vitro*. Cinnamaldehyde was the primary constituent of *C. zeylanicum* essential oil (65.91%). The essential oil of *C. zeylanicum* contains a high level of cinnamaldehyde, which is responsible for its potent antimicrobial properties (Firmino et al., 2018). Cinnamaldehyde ($C_9H_8O$) is an aromatic aldehyde that inhibits the growth of Gram-positive and Gram-negative pathogenic bacteria by destroying the cytoplasmic membrane and causing a decrease in intracellular ATP concentration. Cinnamaldehyde also prevents the growth of fungi such as dermatophytes, filamentous molds, and yeasts (Hamidpour et al., 2015). Cinnamaldehyde can be used as a natural antimicrobial in food preservation to inhibit the growth of pathogenic and spoilage microorganisms, thereby extending the shelf life of food products (Othman et al., 2018). Cinnamaldehyde has been shown to reduce inflammation by inhibiting IL-8 expression in *H. pylori*-infected cells (Muhammad et al., 2015). At a concentration of 2 μg/ml, cinnamaldehyde could inhibit the growth of *H. pylori* after 12 h of incubation (Ali et al., 2005).

## Conclusions

*H. pylori*, the most common human pathogen that colonizes the gastric mucosa, has been linked to peptic ulcers, gastric cancer, and peptic inflammation. Cytotoxic factors, such as *cagA* and *vacA*, contribute to the pathogenicity of *H. pylori*, which remains an intractable challenge in public health around the world. Plant extracts, on the other hand, contain a diverse array of secondary metabolites that have been shown to be effective against *H. pylori* pathogens, such as *C. zeylanicum*. To the best of our knowledge, this is the first report on the biomedical potential of *C. zeylanicum* essential oil against cytotoxin-associated genes that produce drug-resistant *H. pylori*. The most effective anti-*H. pylori* essential oil was determined to be *C. zeylanicum* essential oil. Cinnamaldehyde is the primary active ingredient in *C. zeylanicum* essential oil, which may account for its effectiveness. As a result, *C. zeylanicum* essential oil may be suggested as a source of low-cost precursors for developing novel antimicrobial agents with fewer side effects, particularly in Egypt, where *H. pylori* is prevalent. An additional study is currently being conducted to determine the mechanism by which the purified active cinnamaldehyde inhibits *H. pylori*. This study may provide a useful platform for further investigation of novel derivatives as a new leading structure for biomedical therapeutics against *H. pylori*.

### *Data availability*

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

### *Ethics approval*

All procedures performed in studies involving human participants followed the ethical standards of Tanta University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### *Conflict of interests*

All authors declare no competing interests.

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