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Pomegranate peel extract and *Helicobacter pylori* eradication: an *in vitro* investigation

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Abstract

Helicobacter pylori (*H. pylori*) is a main cause of chronic gastritis, peptic ulcers, and gastric cancer. Despite available treatments, eradication fails in 5–20% of cases, with frequent ulcer recurrence. This highlights the need for alternative therapies, including plant-derived natural products. Therefore, this study aims to evaluate the antibacterial effects of pomegranate peel extracts on *H. pylori* growth. This is a pilot study in which gastric biopsies were collected from *H. pylori*-positive patients at a single

center. Fresh pomegranate (*Punica granatum*) peels were extracted with 80% methanol using ultrasonic-assisted extraction. Following filtration and concentration, extracts were examined for the presence of bioactive compounds. *H. pylori* were grown on brain–heart infusion broth, and the identification was confirmed by evaluating colony morphology, Gram stain, and urease, catalase, and oxidase activity. Antibacterial activity of peel extracts (3–10 milligrams per milliliter) was assessed via inhibition zones, and minimum inhibitory and minimum bactericidal concentrations were determined. Eight gastric biopsies were analyzed. All isolates (100%) were resistant to amoxicillin and amoxicillin-clavulanate, while fully susceptible to levofloxacin and ciprofloxacin, with inhibition zones of 18–29 mm and 18–27 mm, respectively. Pomegranate peel extract was found to exhibit a dose-response relationship in antibacterial activity against tested isolates when used in concentrations of 3, 5, 7, and 10 mg/mL. Inhibition zones ranged from complete inhibition (one isolate) to 24 mm at 3 mg/mL; from complete inhibition (one isolate) to 30 mm at 5 mg/mL; from complete inhibition (one isolate) to 32 mm at 7 mg/mL; and from 15 to 27 mm at 10 mg/mL. Pomegranate could be used as a natural therapeutic agent to establish novel strategies in the prevention of *H. pylori* infection and antibiotic resistance.

Introduction

Helicobacter pylori (*H. pylori*) is one of the most common infectious agents worldwide, infecting approximately half of the world's population, and is a major cause for public health concern.[1] It is a Gram-negative, spiral-shaped bacterium that has evolved a number of adaptive mechanisms that enable it to colonize human gastric mucosa, leading to persistent infection. The infection is a major cause of chronic gastritis, peptic ulcers, and gastric adenocarcinoma in humans.[1] The ability of *H. pylori* to persist in the highly acidic gastric environment, along with its well-established role in carcinogenesis, has led the World Health Organization to categorize *H. pylori* as a group 1 carcinogen.[2] It is estimated that the bacterium is responsible for almost 80% of gastric carcinomas and 5.5% of all human cancers worldwide.[3] The mechanisms of *H. pylori*-induced carcinogenesis are multifactorial and involve a complex interplay between virulence factors, host immunology, and environmental factors, leading to chronic inflammation and damage to the gastric mucosa.[1]

At present, the commonly used protocols for *H. pylori* eradication include antibiotics such as amoxicillin, clarithromycin, metronidazole, and levofloxacin, which are usually used in combination with proton pump inhibitors. The efficacy of such protocols, however, has declined significantly over the years, especially due to the increasing antimicrobial resistance worldwide. Recent studies indicate that only 36.78% of *H. pylori* isolates remain completely susceptible to these agents, while multidrug resistance has been observed among 33.05% of *H. pylori* strains.[4] The prevalence of metronidazole resistance is high, while the increasing rates of resistance against clarithromycin and fluoroquinolones have affected the efficacy of traditional triple therapy in many settings. The resistance of *H. pylori* against such antibiotics is usually caused by chromosomal mutations, although efflux pumps, changes in membrane permeability, biofilm formation also play significant roles in such resistance patterns.[5]

In response to the escalating problem of antibiotic resistance, growing attention has been directed toward natural antimicrobial alternatives. Plant-derived products are especially attractive because of their relatively favorable safety profiles, lower propensity of resistance development, and potential synergistic effects when combined with standard antibiotics.[6] Among these candidates, *Punica granatum* (pomegranate) has received considerable interest owing to its broad spectrum of biological activities. It has been noted that the antioxidant capacity of the peel extract is significantly higher and has anti-inflammatory and anti-ulcerogenic activities that may be ten times stronger than that of the pulp extract.[7] In spite of its widely documented antioxidant activities, its antimicrobial activity, especially against *H. pylori*, has been little explored. The limited number of existing studies on pomegranate peel activity against *H. pylori* have largely relied on standard laboratory strains or isolates from other geographic regions, and few have simultaneously combined bioactive compound characterization with susceptibility testing against clinically derived, antibiotic-resistant isolates. Moreover, to our knowledge no study has evaluated this activity in the context of the high amoxicillin resistance rates documented in the region, where self-medication and uncontrolled antibiotic use represent a significant clinical challenge. Therefore, this study aims to evaluate the antibacterial activity of pomegranate peel extract and to determine its minimum inhibitory and bactericidal concentrations, with the aim of assessing its potential as a natural therapeutic option for *H. pylori* infections. All references were verified for eligibility.[8]

Materials and Methods

Ethical approval

This was a pilot study. All procedures involving human biopsy samples were conducted according to ethical standards for research and after obtaining informed consent from all participants prior to sample collection.

Study design and location

This study was carried out over a period of 12 months from January 2024 to January 2025 in Smart Health Tower, Iraq. Samples of gastric biopsy were collected from patients with histopathologically confirmed *H. pylori* infection. Patients were included if they were adults aged 18 years or older presenting for upper gastrointestinal endoscopy, and had not received antibiotic therapy or proton pump inhibitors in the four weeks prior to biopsy collection. Patients were excluded if they had received prior *H. pylori* eradication therapy, had a history of gastric surgery, or had any condition precluding safe endoscopy. Eligible patients were enrolled consecutively during the study period. As this was a pilot study with no prior local data available to inform a formal power calculation, the sample size of eight was determined by the number of consecutive eligible patients presenting during the study period who met the inclusion criteria and provided informed consent; no a priori sample size calculation was performed. The experimental design, from sample collection to pomegranate peel processing, bioactive compound characterization, *H. pylori* culture, and antimicrobial testing, is shown in Figure 1. All laboratory procedures were carried out in controlled conditions to facilitate reproducibility and reliability.

Plant material preparation

Pomegranate peels of fresh pomegranate fruit, *Punica granatum* L., were obtained, washed with distilled water, and subjected to controlled oven drying. The peels were evenly spread on sterile trays used for drying samples in the laboratory, dried at 40–50°C for 24-48 hours to reach the constant weight for the complete removal of moisture while retaining heat-sensitive bioactive compounds, such as phenolics/flavonoids.

Extract preparation

The dried peels (10g) were mechanically treated with a sterile mortar and pestle. The powder was mixed with 80% high-performance liquid chromatography (HPLC)-grade methanol (100 mL), and then it was treated with ultrasonic extraction using a Branson sonifier (SFX550, Branson Ultrasonics, Danbury, CT, USA), 60% duty cycle for 25 minutes at 50°C. The mixture was then centrifuged at 7,500 rpm for 15 minutes; after that, it was treated with activated charcoal to remove pigments. The filtrate was then reduced to dryness under reduced pressure at 40°C using a Büchi Rotavapor (R-200, BÜCHI Labortechnik AG, Flawil, Switzerland). The dry mixture was then reconstituted in 1.0 mL of 80% HPLC-grade methanol and filtered through a 0.22 µm sterile syringe filter. The final mixture was then stored at 4°C in amber vials before analysis.

Identification of bioactive compounds (flavonoids) in **Punica granatum**

Gas Chromatography–Mass Spectrometry (GC–MS)

GC-MS (Shimadzu GCMS-QP series, Shimadzu Corporation, Kyoto, Japan) equipped with an Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used to determine the chemical profile of *Punica granatum* peel extract. The extract, filtered through a 0.22 µm membrane filter, was injected (1 µL; split mode) with helium as the carrier gas (1.5 mL/min). The oven temperature program began at 60°C (2 min hold), increased at 10°C/min to 280°C, and held for a total runtime of 40 minutes.

Mass spectra were obtained in electron impact mode (70 eV) with a scan range of 50–600 m/z; injector and detector temperatures were set at 250°C. Compound identification was done by matching with National Institute of Standards and Technology (NIST 20, National Institute of Standards and Technology, Gaithersburg, MD, USA) and the Wiley library spectra (John Wiley & Sons, Hoboken, NJ, USA) and retention times and fragmentation patterns to authentic standards. The analysis revealed the presence of several furan derivatives, organic acids, aldehydes, and phenolic compounds, especially flavonoids and other antioxidant molecules (Figure 2). The relative abundance of each individual compound was calculated in percentage based on the total peak area in the chromatogram, hence creating a comprehensive chemical fingerprint of the extract.

High-performance liquid chromatography

Phenolic content in the extract was analyzed qualitatively and quantitatively by HPLC coupled with a photodiode array detector (PDA) (Shimadzu Corporation, Kyoto, Japan, LabSolutions), with detection performed at 280 nm. The analysis was carried out using a C18 reverse-phase column (250 × 4.6 mm, 5 µm particle size) with gradient elution program consisting of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), with a flow rate of 1.0 mL/min, injection volume of 20 µL, and ambient column temperature.

Phenolic standards (pyrogallol, gallic acid, caffeic acid, chlorogenic acid, ferulic acid, ellagic acid, cinnamic acid, salicylic acid, coumaric acid; Sigma-Aldrich, St. Louis, MO, USA, 0.02–0.14 mg/mL in methanol) were employed for the calibration of retention times and peak areas. Identification of sample was done by matching retention times with those of standards. Quantification was done by comparing the peak area of each compound in the sample with that of its standard at a known concentration, using a dilution factor. The concentration of each compound was calculated using the following equation:

$$\text{Sample Concentration} = \frac{\text{Sample Peak Area (} \times \text{ dilution factor)}}{\text{Standard Peak Area}} \times \text{Standard Concentration}$$

All chromatographic data were analyzed using Shimadzu LabSolutions software (Shimadzu Corporation, Kyoto, Japan). Results were reported as mg/mL of each phenolic compound in the extract. Major and minor phenolic compounds were identified on the basis of relative peak area and concentration.

H. pylori *culture and standardization*

Fresh gastric biopsies were immediately transferred into Brain–Heart Infusion (BHI) broth, which served as the transport medium. The biopsy tissue was homogenized in BHI using a sterile homogenizer to provide an even distribution of the sample. The homogenized sample was then transferred into 20 mL of fresh BHI broth, which functioned as the isolation and growth medium. Cultures were incubated in a shaker incubator at 37°C for 24–48 hours. Successful bacterial growth was indicated by the turbidity of the broth.

After growth, *H. pylori* isolates were confirmed based on colony characteristics after subculture, Gram staining (characteristic spiral-shaped Gram-negative bacteria), and positive results for urease, catalase, and oxidase tests. Pure cultures were standardized by resuspending bacterial cells in sterile BHI broth and adjusting turbidity to a McFarland 3.0 standard (~10^9 Colony Forming Units - CFU/mL) using a spectrophotometer (GENESYS 10S, Thermo Fisher Scientific, Madison, WI, USA). Working suspensions of 10^6 CFU/mL were prepared for antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

Pomegranate peel extract concentrations of 3 mg/mL, 5 mg/mL, 7 mg/mL, and 10 mg/mL were prepared in sterile dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and added into BHI blood agar plates. DMSO alone, at the equivalent concentration used in the extract preparations, was applied to separate plates as a negative control to confirm that the solvent itself had no bacteriostatic or bactericidal effect on *H. pylori* at the tested concentrations. Standardized suspensions of *H. pylori* (10^6 CFU/mL) were spot inoculated onto the plates containing the extract. All plates were incubated for 72 hours at 37°C under microaerophilic conditions. The zones of inhibition were measured using digital calipers.

Minimum inhibitory concentration and minimum bactericidal concentration

A stock solution of the extract was prepared at a concentration of 100 mg/mL in DMSO. For the serial two-fold dilution, 2 mL of sterile Brucella broth (HiMedia Laboratories, Mumbai, India) containing 10% fetal bovine serum was added to five sterile test tubes labeled 1 to 5. An additional 2 mL of the stock solution of the extract was added to Tube 1, giving a final volume of 4 mL and a final concentration of 50 mg/mL. Serial two-fold dilution of the extract was performed by transferring 2 mL of the solution from Tube 1 to Tube 2, mixing well, and repeating the same for the other tubes up to Tube 5, giving final concentrations of 50, 25, 12.5, 6.25, and 3.125 mg/mL.

Each of the tubes was inoculated with 0.2 mL of a standardized *H. pylori* suspension (containing 10^6 CFU/mL, equivalent to McFarland 3.0) and incubated at 37°C for 72 hours in a microaerophilic environment using a gas-generating system. The minimum inhibitory concentration (MIC) was

determined as the lowest concentration of the extract that showed no visible bacterial growth, i.e., no bacterial turbidity after 72 hours of incubation.

For the determination of the minimum bactericidal concentration (MBC), 20 µL of the bacterial cultures in tubes that showed no growth were sub-cultured onto BHI blood agar plates containing 5% sheep blood and selective antibiotics. The MBC was defined as the lowest concentration of the extract at which no bacterial growth was visible. *H. pylori* American Type Culture Collection (ATCC) 43504 was used as a reference strain, and the standard antibiotics (amoxicillin, amoxicillin-clavulanate, and levofloxacin) were tested simultaneously as controls, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Ciprofloxacin, a fluoroquinolone not routinely used in standard *H. pylori* eradication regimens, was additionally included to assess potential intra-class cross-resistance patterns between fluoroquinolones, given the local clinical relevance of this antibiotic class.

Results

In this study, 8 biopsy samples were included. HPLC analysis of the extract showed the presence of several phenolic compounds, with salicylic acid (16.76 mg/mL), pyrogallol (12.02 mg/mL) and chlorogenic acid (0.414 mg/mL) being present at relatively higher amounts (Table 1). The eight isolates of *H. pylori* were found to be resistant to both amoxicillin and amoxicillin-clavulanate, accounting for 100% resistance. However, the isolates were found to be susceptible to fluoroquinolones. The inhibition zones for levofloxacin were found to be 18 to 29 mm, while for Ciprofloxacin, it was between 18 and 27 mm, indicating 100% susceptibility to both antibiotics (Table 2).

The antibacterial activity of the extract was tested at concentrations of 3 mg/mL, 5 mg/mL, 7 mg/mL, and 10 mg/mL. At a concentration of 3 mg/mL, the inhibition zones ranged from complete inhibition of bacterial growth to 24 mm. At 5 mg/mL concentration, the inhibition zones ranged from complete inhibition of bacterial growth to 30 mm. At a concentration of 7 mg/mL, the inhibition zones ranged from complete inhibition of bacterial growth to 32 mm. At the highest concentration, 10 mg/mL, all isolates showed inhibition, with zones ranging from 15 mm to 27 mm. The MIC values ranged from 6.25 to 12.5 mg/mL, while the MBC values ranged from 12.5 to 25 mg/mL, indicating bacteriostatic activity at lower concentrations and bactericidal effect at higher concentrations (Table 3).

Discussion

H. pylori is known to be a major causative agent of peptic ulcer disease in both adults and children. Although successful treatment regimens are available, antibiotic resistance in many patients leads to treatment failure.[9] Thus, it is imperative to investigate both synthetic and natural alternatives to alleviate the problem of antibiotic resistance in *H. pylori*.[9]

The main antibiotics that have been targeted in the eradication of *H. pylori* are clarithromycin and levofloxacin. Both these drugs have potent bactericidal activity against susceptible strains and thus are essential components of the current eradication regimens. In 2017, the World Health Organization classified *H. pylori* as a high-priority target for antibiotic research due to the rapid increase in the incidence of clarithromycin resistance worldwide.[10] However, in 2024, in a surprising move, clarithromycin was removed from the priority list, despite the continued increases in resistance in most countries.[11] Amoxicillin is still a mainstay in both triple and dual therapy. High-dose proton pump inhibitor therapy, together with amoxicillin, has shown high efficacy, and the addition of potassium-competitive acid blockers further improves acid suppression and eradication success.[10] The increasing prevalence of resistance to levofloxacin, which is recommended as second-line therapy in Europe, most of Asia (except Japan, where sitafloxacin is preferred), and Africa, limits its use and thus underscores the need for antimicrobial susceptibility testing-guided therapy.[12-15] A key limitation of dual therapy is the rising prevalence of amoxicillin resistance, which exceeds 70% in some African countries and varies from 15% to 50% in several Asian countries, including Vietnam and Iran.[10]

An international survey was carried out in 31 countries across six continents between 2018 and 2023 to investigate the global distribution of *H. pylori* antibiotic resistance, and the results showed large geographic variation. At the continental level, Africa had high resistance rates, with ciprofloxacin resistance ranging from 17% to 100%, levofloxacin resistance ranging from 20% to 65.7%, and amoxicillin resistance up to 97.1%. However, North and South America had relatively lower resistance rates, with levofloxacin resistance ranging from 13.5% to 42.6% and amoxicillin resistance ranging from 1.1% to 2.7%. In the Asia-Pacific region, resistance rates were 37.0% for ciprofloxacin, 3.3%–65.6% for levofloxacin, and 0%–50% for amoxicillin, indicating substantial inter-regional variations.[10] Country-wise data also supported these findings. In Egypt, the resistance rate of amoxicillin was alarmingly high (81.9%–95%), while the resistance rates of levofloxacin and ciprofloxacin were lower

(≈20% and 4.7% to 17%, respectively). Ethiopia had 91.7% resistance to amoxicillin and 66.7% resistance to ciprofloxacin. However, in France the resistance rates were very low, with levofloxacin resistance ranging from 17.6% to 18.1% and amoxicillin resistance at 0%.[10] In the present study, all tested *H. pylori* isolates demonstrated resistance to amoxicillin, while resistance to levofloxacin and ciprofloxacin was absent. This unusual finding can be explained by overuse of antibiotics in Iraq, including their uncontrolled use and self-medication. Such practices can create strong selective pressure, which can foster the emergence of resistant strains, especially for commonly used antibiotics such as amoxicillin.[16]

Pomegranate peel, which is the by-product of *Punica granatum* L. fruit, contributes to 50% of the weight of the entire fruit. Pomegranate peel contains bioactive compounds such as ellagic acid, phenolic acids, and flavonoids, which exhibit different biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-apoptotic, and cell regeneration properties.[17]

The antibacterial properties of pomegranate peel are mainly due to the high concentration of phenolic compounds, which have the potential to prevent or reduce infections. These compounds work by precipitating membrane proteins and interfering with crucial enzymatic reactions, resulting in the death of bacterial cells, thereby contributing to the antimicrobial properties of pomegranate peel.[18] Al-Zoreky demonstrated that pomegranate peel extract has a significant inhibitory effect on *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Yersinia pestis* in small intestinal colitis models.[19] Panichayupakaranant *et al*. reported that pomegranate peel extract containing 13% (w/w) ellagic acid has strong antibacterial activity. Moreover, it has been demonstrated that a concentration of 2 mg/disk of pomegranate peel extract effectively inhibits Gram-positive bacteria such as *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Because of these properties, plant extracts have been used to a greater extent for the development of bioactive packaging materials.[20] Hanani *et al*. carried out a comparative study on antibacterial activity using pomegranate, papaya, and pineapple peel extracts. The results showed that only pomegranate peel extract-loaded membranes could effectively inhibit all strains of bacteria, including *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella enterica* serovar Typhimurium.[21]

There have been some *in vitro* and *in vivo* studies to evaluate the effects of pomegranate peel on *H. pylori*. In one such study, the ethanol extract of the pomegranate peel was examined for its efficacy on the standard strain of *H. pylori* National Collection of Type Cultures (NCTC)-11916 by *in vitro* and *in*

vivo experiments in female Wistar rats. The pomegranate peel ethanol extract was shown to be safe and had strong antibacterial properties with a MIC of 0.156 mg/mL, along with strong urease inhibition ($IC_{50} \approx 6$ mg/mL). A synergistic effect was also observed with metronidazole, and oral treatment for eight days resulted in a significant decrease in *H. pylori*-induced gastritis and lymphocytic infiltration and chronic inflammation.[7] Another study that evaluated the efficacy of medicinal plant extracts and bacteriophages included 35 gastric biopsies, of which 10 (28.6%) were culture-positive. Among the tested extracts, *Punica granatum* peel extract was found to be most effective *in vitro*.[22] *In vivo* studies in rats using the oral route showed significant reduction in bacterial load ($p < 0.05$) and ulcer healing. Other plants such as *Rosmarinus officinalis*, *Syzygium aromaticum*, *Rhus coriaria*, and *Ammi visnaga* also showed inhibitory effects. Though sewage water screening revealed the presence of three *H. pylori*-specific bacteriophages, the infectivity was lost during purification, thus reducing the usefulness of phage therapy. In general, the efficacy of *Punica granatum* peel extract was better than the conventional antimicrobial regimens, indicating its potential as an adjunct treatment for *H. pylori* infection.[22] Furthermore, the antimicrobial and urease-inhibitory activities of pomegranate peel and licorice extracts, fractionated using ethyl acetate and methanol into four fractions (F1–F4), were also investigated against *H. pylori*. All fractions exhibited antimicrobial activity, with the F1 fractions of both pomegranate peel and licorice being most active, producing zones of inhibition of 33 mm and 26 mm, and MICs of 2 mg/mL and 1 mg/mL, respectively. The remaining fractions were moderately active, with MIC values of 4 to 8 mg/mL. This indicates that both pomegranate and licorice contain highly active bioactive agents against *H. pylori* and hence should be explored further.[23] The MIC values observed in this study (6.25–12.5 mg/mL) exceed those reported by Mayyas *et al.* (0.156 mg/mL); however, direct comparison should be interpreted cautiously due to methodological differences between the studies.[7] The extract here underwent a 100-fold concentration step, so the reported values reflect a highly concentrated preparation, and the equivalent unconcentrated concentrations would be considerably lower. In addition, earlier studies commonly employed standard laboratory *H. pylori* strains (e.g., ATCC or NCTC), whereas this study examined clinical isolates with complete amoxicillin resistance, which are inherently more difficult to inhibit. Variations in extraction solvents, fractionation approaches, and susceptibility testing methods (e.g., agar dilution vs. broth microdilution) further complicate direct comparisons. Notably, all strains of *H. pylori* were resistant to amoxicillin and amoxicillin/clavulanic acid. In contrast, all fractions of pomegranate peel extract were found to exhibit antibacterial activity, and the fraction containing 10 mg/mL was found to exhibit the most potent activity, with zones of inhibition >15 mm in diameter. These findings suggest that

pomegranate peel could represent a promising alternative candidate for the management of antibiotic-resistant *H. pylori* infections.

This study has certain limitations. This *in vitro* study design does not take into account the complex physiological environment *in vivo*, in which factors such as gastric acidity and mucosal defense mechanisms may play a role in the antibacterial activity. Additionally, the limited number of clinical isolates used in the study (n = 8) may impact the overall generalizability of the study, and the absence of a direct comparison to standard antibiotics may impact the ability to fully assess the relative effectiveness of the extract. The small sample size reflects the exploratory, hypothesis-generating nature of this pilot study, in which no prior local data were available to support a formal power calculation. Future adequately powered, multicenter studies with a larger and more diverse cohort of clinical isolates will be necessary to confirm these preliminary findings, establish the true local prevalence of antibiotic resistance, and provide a more robust assessment of the antimicrobial efficacy of pomegranate peel extract. It should also be noted that different culture media were used across the two antimicrobial assays: BHI blood agar for inhibition zone measurements and Brucella broth supplemented with fetal bovine serum for MIC/MBC determination. This reflects standard microbiological practice, as Brucella broth is the recommended medium for *H. pylori* broth microdilution susceptibility testing according to EUCAST guidelines, while BHI blood agar supports robust colony growth for disc/spot diffusion assays. Nevertheless, the differing nutritional compositions of these media may influence bacterial growth kinetics and extract bioavailability, and direct quantitative comparisons between inhibition zone results and MIC/MBC values should be interpreted with appropriate caution. Despite these limitations, this study offers important preliminary data regarding the potential for the antibacterial activity of pomegranate peel extract against *H. pylori* and serves as a valuable starting point for further *in vivo* and comparative studies aimed at validating and extending the results.

Conclusions

Pomegranate peel extract may exhibit promising antibacterial properties against *H. pylori*, including resistant bacterial strains, suggesting its potential as a natural therapeutic agent pending further *in vivo* validation. The bioactive compounds in the pomegranate peel could contribute to the development of new strategies in the management of *H. pylori* infections.

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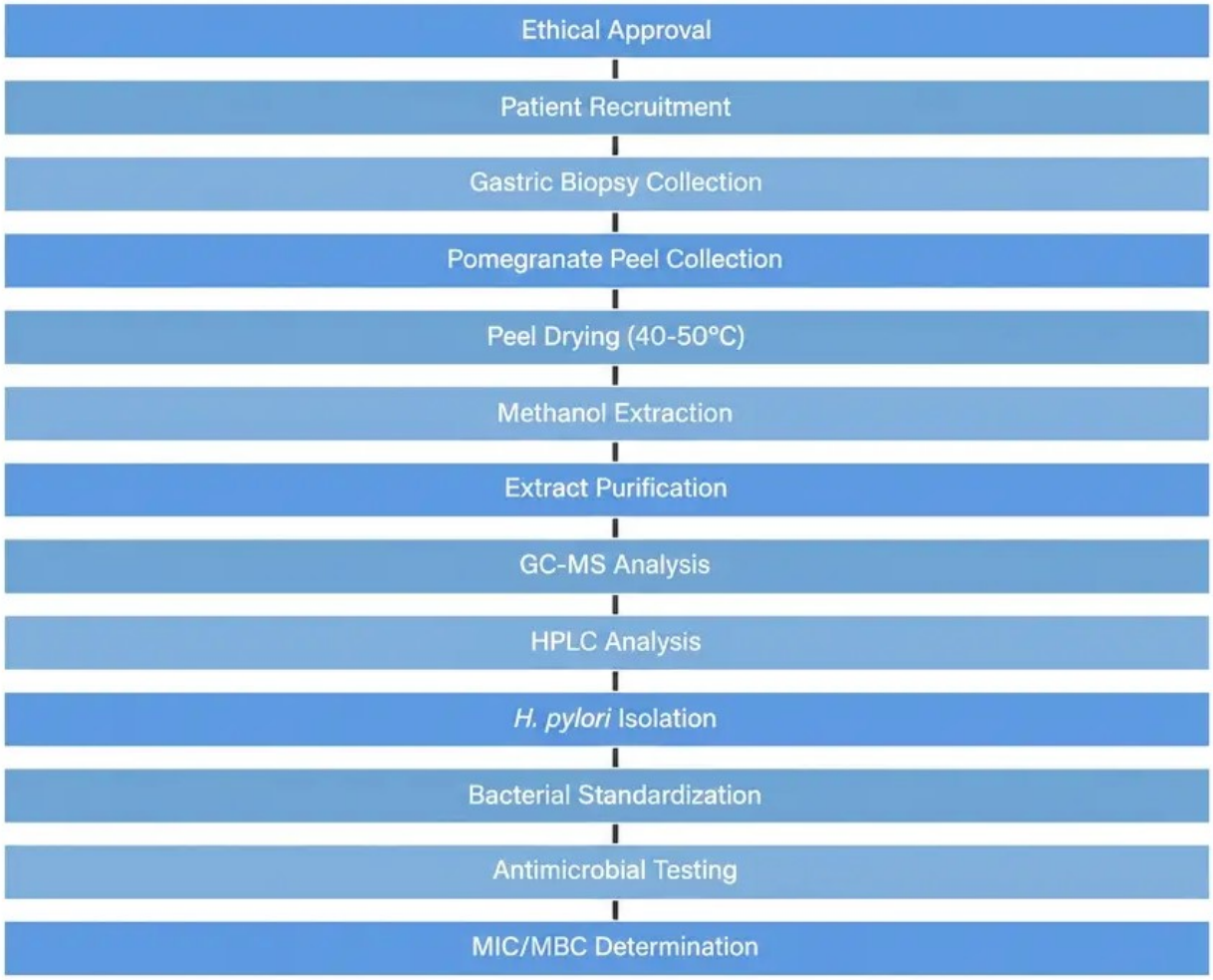
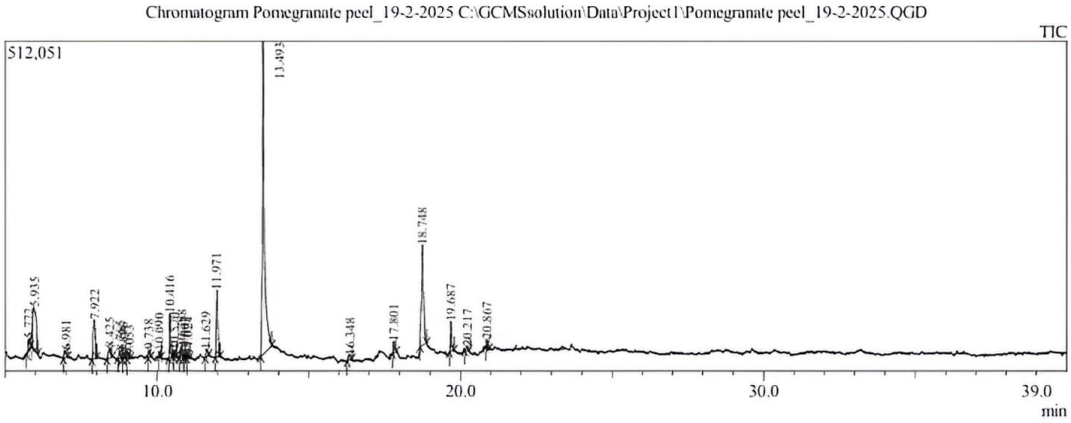


Figure 1. Study flow chart illustrating the study workflow, including ethical approval, patient recruitment, gastric biopsy collection, pomegranate peel collection and processing, methanolic extract preparation, chemical characterization by gas chromatography–mass spectrometry (GC–MS) and High-Performance Liquid Chromatography (HPLC), *Helicobacter pylori* (*H. pylori*) isolation and standardization, antimicrobial susceptibility testing, and determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Sample Type : Food
Sample Name : Pomegranate peel
Sample ID : 5
Sample Amount : 1 ul
Vial # : 1.5 ml
Injection Volume :1 ul
Method File : 40 min



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	5.772	5.700	5.825	67856	1.23	16417	1.41	4.13	MI	Ethanedioic acid, dimethyl ester
2	5.935	5.858	6.067	518621	9.41	64552	5.54	8.03	MI	Furfural
3	6.981	6.908	7.058	58290	1.06	11068	0.95	5.27	MI	2-Propanone, 1,3-dihydroxy-
4	7.922	7.850	8.008	277178	5.03	60526	5.19	4.58	MI	2,5-Furandione, dihydro-3-methyle
5	8.425	8.342	8.525	88432	1.60	16090	1.38	5.50	MI	2-Furancarboxaldehyde, 5-methyl-
6	8.765	8.700	8.842	37764	0.69	10960	0.94	3.45	MI	2,4-Dihydroxy-2,5-dimethyl-3(2H)
7	8.896	8.858	8.958	26155	0.47	8685	0.75	3.01	MI	3-Furanol, tetrahydro-
8	9.053	9.008	9.108	13684	0.25	4420	0.38	3.10	MI	Acetic acid, trichloro-, octyl ester
9	9.738	9.700	9.800	34286	0.62	11765	1.01	2.91	MI	1,4-Dioxin, 2,3-dihydro-5,6-dimeth
10	10.090	10.058	10.142	22297	0.40	8096	0.69	2.75	MI	Oxalic acid, cyclobutyl nonyl ester
11	10.416	10.375	10.508	203617	3.69	69048	5.92	2.95	MI	Acetic acid, 1-(2-methyltetrazol-5-y
12	10.570	10.533	10.633	28004	0.51	9277	0.80	3.02	MI	6-Methyl-2-pyrazinylmethanol
13	10.788	10.725	10.842	51728	0.94	16550	1.42	3.13	MI	Methyl 2-furoate
14	10.907	10.867	10.950	12911	0.23	5197	0.45	2.48	MI	Hexadecane
15	11.024	10.975	11.067	16908	0.31	5311	0.46	3.18	MI	Acetic acid, 2-(N-methyl-N-phosph
16	11.629	11.583	11.708	37390	0.68	11149	0.96	3.35	MI	Pentanoic acid, 4-oxo-
17	11.971	11.908	12.067	341485	6.20	104181	8.94	3.28	MI	4H-Pyran-4-one, 2,3-dihydro-3,5-d
18	13.493	13.417	13.783	2485518	45.10	485905	41.69	5.12	MI	2-Furancarboxaldehyde, 5-(hydrox
19	16.348	16.258	16.425	44347	0.80	7953	0.68	5.58	MI	4-Pentenoic acid, 3-hydroxy-, ethyl
20	17.801	17.758	17.892	70960	1.29	17814	1.53	3.98	MI	Furazan-3-ol, 4-amino-
21	18.748	18.675	18.892	840247	15.25	155955	13.38	5.39	MI	Sucrose
22	19.687	19.642	19.792	162070	2.94	46647	4.00	3.47	MI	.beta.-D-Glucopyranose, 1,6-anhyd
23	20.217	20.142	20.283	28916	0.52	6484	0.56	4.46	MI	.beta.-D-Lyxofuranoside, 5-O-(.bet
24	20.867	20.833	20.950	42392	0.77	11596	0.99	3.66	MI	1,6-Anhydro-.alpha.-d-galactofuru
				5511056	100.00	1165646	100.00			

Figure 2. Gas Chromatography–Mass Spectrometry (GC–MS) chromatogram of the methanol extract of *Punica granatum* (pomegranate) peel.

Table 1. Quantitative analysis of phenolic compounds identified in the methanol extract of pomegranate peel using High-Performance Liquid Chromatography (HPLC)

No.	**Compounds**	**RT (ST)**	**Area (ST)**	**Standard Con. (mg/mL)**	**RT (Sample)**	**Area (Sample)**	**Area (San (20) df**

1	Salicylic acid	10.101	7466	0.1	9.905	62584	1251680
2	Pyrogallol	5.033	114612	0.02	4.853	3442945	68858900
3	Chlorogenic acid	13.829	21212	0.1	13.736	4395	87900
4	Ferulic acid	13.176	5159	0.1	12.702	698	13960
5	Caffeic acid	12.168	1349524	0.14	12.250	15106	302120
6	Coumaric acid	9.043	534968	0.02	8.912	17572	351440
7	Ellagic acid	13.218	9795637	0.14	13.361	28825	576500
8	Gallic acid	12.014	1183464	0.1	11.888	2635	52700
9	Cinnamic acid	11.164	3494857	0.1	10.822	2962	59240

df, dilution factor; RT, retention time; ST, Standard; Con., Concentration. Concentrations are expressed as mg/mL of the final reconstituted extract, prepared by evaporating 100 mL of methanol extract to dryness and reconstituting in 1.0 mL of 80% methanol (100-fold concentration factor); values are equivalent to mg per 10 g dry peel per mL of final solution.

Table 2. Antibiotic susceptibility of *H. pylori* isolates determined by inhibition zone.

Patient ID	Amoxicillin (mm)	Amoxicillin-clavulanate (mm)	Levofloxacin (mm)	Ciprofloxacin (mm)
6	R	R	S (25)	S (27)
8	R	R	S (18)	S (22)
18	R	R	S (20)	S (18)
26	R	R	S (20)	S (18)
11	R	R	S (29)	S (24)
25	R	R	S (21)	S (21)
29	R	R	S (24)	S (25)
30	R	R	S (22)	S (24)

R, Resistant; S, Sensitive; mm, millimeter of inhibition zone.

Table 3. Plant extract antimicrobial activity against clinical isolates.

Patient ID	3 mg/mL	5 mg/mL	7 mg/mL	10 mg/mL	MIC	MBC
1	No growth	No growth	No growth	22	6.25	12.5
2	20	20	20	20	12.5	25
3	6	6	6	18	12.5	25
4	10	12	12	20	6.25	12.5
5	10	10	12	17	12.5	25
6	24	30	32	27	12.5	25
7	15	15	15	15	12.5	25
8	13	14	13	15	12.5	25
Mean ± SD†	14.0 ± 6.2	15.3 ± 7.8	15.7 ± 8.3	19.2 ± 4.0	-	-

MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration.

†Mean ± Standard Deviation (SD) calculated for measurable inhibition zones only (n=7 for 3, 5, and 7 mg/mL; n=8 for 10 mg/mL). Patient 1 exhibited complete inhibition (no growth) at 3, 5, and 7 mg/mL, representing the strongest antimicrobial response; this categorical outcome was excluded from mean ± SD calculations as it cannot be expressed as a numerical zone diameter

Contributions: Mariwan Fathalla Abdalfatah, Namam Rebaz Mohammed, and Lalan Rebaz Mohammed made major contributions to the conception and design of the study and conducted the

literature search. Shena Salam Omer, Aynda Sdiq, Ayman M. Mustafa, and Fahmi H. Kakamad contributed to the literature review and drafting of the manuscript. Dana T. Gharib, Hoshmand R. Asaad, Sivan H. Salih, and Karokh F. Hama Hussein contributed to the literature review, study design, critical revision of the manuscript, and preparation of the tables and figures. Ayman M. Mustafa and Fahmi H. Kakamad verified the authenticity of all raw data. All authors reviewed and approved the final manuscript.

Conflict of interest: the authors declare that they have no conflict of interest.

Ethics approval and consent to participate: ethical approval for this study was obtained from the Ethical Committee of Kscien Organization for Scientific Research (Approval No. 42; December 20, 2023).

Consent to participate and consent for publication: Written informed consent was obtained from the patients for the publication of their anonymized data.

Availability of data and materials: the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Funding: no funding was received.

AI use statement: the authors declare the use of AI (ChatGPT 5.2) to rephrase and enhance the clarity of the text of the manuscript. All scientific content, critical analysis, and conclusions are entirely original and remain the full responsibility of the authors, who verified and approved every part of the manuscript. No sections were autonomously generated by AI.