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Synthesis, Characterization, and Evaluation of Anti-Helicobacter Activity of Chitosan and Pectin Microparticles Containing *Zataria multiflora* Extract *In Vitro*

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Abstract

Background and Objective: *Helicobacter pylori* infection is of widespread diseases in the world. The most common treatment for remediation of its symptoms is administration of antibiotics, which are not efficient enough in some patients and resulted in antibiotic resistance. Given the adverse effects arising from antibiotics in clinical studies, we aimed to fabricate chitosan- and pectin-based micro-particles containing *Zataria multiflora* extract for suppression of *Helicobacter pylori* in the laboratory.

Materials and Methods: Chitosan and pectin micro-capsules alone and in combination with *Zataria multiflora* extract were prepared by spray dryer. The powders were further characterized by FT-IR, zeta sizer, and scanning electron microscope. Simulated gastric fluid was also prepared for evaluation of anti-Helicobacter potency of the samples.

Results and Conclusion: Chitosan 1% w v^{-1} + 0.5 ml *Zataria multiflora* extract ($CE_{0.5}$), pectin 1% w v^{-1} + 0.3 ml *Zataria multiflora* extract ($PE_{0.3}$), and pectin 1% w v^{-1} (P_1) could significantly suppress the bacteria under simulated gastric condition. The least survivability of *Helicobacter pylori* was 45.4% and achieved for $CE_{0.5}$, followed by 45.70% and 46.6% for $PE_{0.3}$ and P_1, respectively. Electrostatic charge of the biopolymers and phenol compounds of the extract greatly affected the integrity of bacterial cell wall. According to FT-IR spectra, *Zataria multiflora* extract was physically entrapped in chitosan and pectin layers, which helped its better antimicrobial activity *in vitro*. Regarding the significant anti-Helicobacter activity of our selected formula, they would be considered as complementary treatment for antibiotics in eradication of *Helicobacter pylori* infection. Although further *in vivo* experiments are required to validate the current findings.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Helicobacter pylori (*H.) pylori* infection is a wide-spread disease affecting about 50% of people in the world, which may lead to sever diseases such as stomach cancer [1,2]. This bacterial pathogen was introduced as class I carcinogen by IARC in 1994 [3] and disturbs normal func-tion of human digestive system by suppressing absorption of some micronutrients [4]. The most common medication for eradication of *H. pylori* is mixture of two antibiotics and a proton pump inhibitor at different doses. However, some of curing regimens have shown insufficient impact on *H. pylori*-infected patients and have led to antibiotic resistance [1,5].

There are several species of thyme belonging to the *Labiatae* family in Iran. They have potential health effects of antimicrobial, antioxidant, and anti-inflammatory in human owing to the activity of their aromatic compounds.

Thymol, *p*-cymene, and carvacrol are the prominent components in thyme extract [6,7]. These chemicals kill bacteria by affecting normal function of cell membranes, decreasing adenosine triphosphate concentration within the cells, and inhibiting bacterial adherence to epithelial cells [8,9]. With regard to the anti-Helicobacter activity, Korona-Glowniak et al. investigated nine herbal essential oils of which *Thymus vulgaris* EO could significantly decrease *H. pylori* population by inhibition of its urease activity at higher concentrations *in vitro* [4]. Chemical composition of essential oils and herbal extracts depends on geographical distribution and species of the plants they were originated from, which affect their health promotion activity [10]. *Zataria multi-flora* is one of endemic species of thyme grown in south of Iran [6]. Its antimicrobial activity against Gram-positive and Gram-negative bacteria has been reported by several studies [11-13]. However, it alone is not efficient enough in suppression of *H. pylori* [5]. Considering the high number of people suffering from *H. pylori* infection globally and inefficiency of common medications, we tried to increase the antimicrobial potency of *Z. multiflora* by using other natural bioactive compounds. It has been reported that charged biopolymers have antimicrobial impact on a wide range of microorganisms. They are popular in biomedicine because are nontoxic and biodegradable [14].

Chitosan, as a cationic biopolymer, is composed of β-1,4-glucosamine and β-1,4-N-acetyl glucosamine residues. It represses bacterial growth at different levels depending on its zeta potential, molecular weight, and size. Interestingly, Luo et al. observed inhibitory effect of chitosan nanoparticles against *H. pylori* in the laboratory and the antimicrobial activity increased by size reduction and decreasing acetylation degree of chitosan [15]. Chitosan microsphere was also prepared by Goncalves et al. to evaluate its anti-Helicobacter activity. They used genipin as a less toxic cross-linker compared to other chemicals. The authors tried to decrease solubility and maintain mucosal adhesion ability of the cross-linked chitosan under gastric condition, through which the polymer attached to the epithelial surface and *H. pylori*, simultaneously. Interestingly, chitosan microsphere could decrease colonization of *H. pylori* on the epithelium surface in a model system [16]. Chitosan can be used together with antibiotics in treatment of bacterial infection by reducing the required concentration of antibiotics. In this regard, Nguyen et al. observed three-fold inhibitory impact of chitosan-amoxicillin compared to amoxicillin alone in complete suppression of *Streptococcus pneumonia* [17].

Antimicrobial activity of pectin or its derivatives have been reported by several studies [18,19]. It is anionic polysaccharide extracted from different plants especially apple and citrus [20]. Daoud et al. reported the antimicrobial impact of citrus pectin on 16 clinical isolates and two commercial strains of *H. pylori* [21]. Although its underlying mechanism of action is not clear, it has been introduced as a natural antimicrobial agent and a comple-mentary medication for bacterial infections.

With respect to the approved antimicrobial activity of thyme extract, chitosan, and pectin, we examined their potential synergism in the laboratory for the first time. For this purpose, the authors fabricated microcapsules of chitosan and pectin loaded with *Z. multiflora* extract by spray dryer. Our objective was protection of the extract in the environment by the antimicrobial biopolymers to evaluate their synergism in *H. pylori* suppression under simulated gastric condition.

2. Materials and Methods

2.1. Materials

Z. multiflora extract (Barij essence Co., Iran), chitosan (Deacetylation degree >75%, Sigma Aldrich, USA), apple pectin (high methoxy, Sigma Aldrich, USA), and acetic acid (Merck, Germany) were used for preparation of micro-particles. *H. pylori* clinical isolates were provided from Helicobacter Research Department at Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, and resuscitated in Brain Hear Infusion media (Merck, Germany) containing Fetal Calf Serum (Iran). Brucella agar (Merck, Germany), Campylobacter supplement III (Skirrow, Merck, Germany), Fetal Calf Serum (Iran), Sheep blood (Iran), and amphotericin B (Merck, Germany) were used for enumeration of *H. pylori* in the laboratory. Urea (Merck, Germany), hydrochloric acid (Sigma Aldrich, USA), and pepsin (Aria Kimia Gostar Co., Iran) were used for preparation of simulated gastric juice.

2.2. Sample preparation

Concentration of the polymers and the extract was determined based on our preliminary study. Chitosan solution (1% w v^{-1}) in 1% v v^{-1} acetic acid was prepared at ambient temperature under stirring at 950 rpm until a homogenous solution was achieved. Pectin solution (1% w v^{-1}) was prepared by addition of 10 g pectin to 1000 ml distilled water followed by stirring at 950 rpm at 45 °C to achieve a clear solution. Amounts of 0.3 and 0.5 ml *Z. multiflora* extract were separately added to the polymeric solutions. The final mixtures were left overnight in refrigerator for complete hydration. Then, each solution was dried by spray dryer with nozzle tip hole of 0.7 mm (Datis energy Co., Iran) at 150 °C by flow rate of 2 ml min^{-1}. Eight samples of chitosan 1% w v^{-1} (C_1, dried powder), chitosan 1% w v^{-1} + 0.3 ml *Z. multiflora* extract ($CE_{0.3}$, dried powder), chitosan 1% w v^{-1} + 0.5 ml *Z. multiflora* extract ($CE_{0.5}$, dried powder), pectin 1% w v^{-1} (P_1, dried powder), pectin 1% w v^{-1} + 0.3 ml *Z. multiflora* extract ($PE_{0.3}$, dried powder), pectin 1% w v^{-1} + 0.5 ml *Z. multiflora* extract ($PE_{0.5}$, dried powder), and *Z. multiflora* extract alone at concentration of 1.5 and 2.5 µl were used for microbial survivability test.

2.3. Sample characterization

Morphology of the spray-dried powders was evaluated by scanning electron microscope (model DSM-960A, Zeiss, Germany) equipped with SE detector. The most effective samples in suppression of *H. pylori* were selected for this purpose. The samples were coated by gold and were graphed under vacuum by acceleration voltage of 30 kV and ×5000 magnification. Chemical interaction of the compon-ents was determined by Fourier transform infrared spectrometer

(Perkin Elmer Spectrum One, Waltham, MA, USA) by recording the transmittance (%) of the waves in range of 4000-450 cm^{-1}. The samples were firstly mixed with KBr and further inserted in the instrument in form of tablet. Owing to the ionic nature of the biopolymers, net charge of each sample was determined by zeta-sizer instrument (SZ-100, Horiba Scientific, Japan) at 25 °C.

2.4. Simulated gastric juice

Simulated gastric medium was prepared according to the method of Gutierrez-Zamorano et al. with some modifications [22]. At first, 0.08 g pepsin was dissolved in 1 ml HCl solution (0.1 M). Then, 0.5 ml of the pepsin solution was added to a test tube. In the next step, 1.2 µl of urea solution (10 g urea in 10 ml distilled water) was added to the tube and the mixture was made up to 10 ml by saline solution (0.9% w v^{-1}). pH of the medium was adjusted to 3.

2.5. H. pylori survivability test

Approximately, 50 mg of each spray-dried powder, 1.5 µl, and 2.5 µl of *Z. multiflora* extract was separately added to 10 ml of the simulated gastric juice. Before bacterial inoculation in the simulated medium, the species were isolated from stock solution and cultured in brain heart infusion medium. The inoculated medium was incubated at 37 °C for 3-5 days under microaerophilic atmosphere containing 85% N_2, 10% CO_2, and 5% O_2. After resuscitation, turbidity of the microbial suspension was adjusted to McFarland standard 2 (approximately equals 10^6 CFU ml^{-1}). At the end, 10 ml of the adjusted bacterial suspension was transferred to 10 ml simulated juice containing each sample. Before incubation, 500 µl of each media was transferred to microplates in duplicate. They were incubated at 37 °C for 2 h under microaerophilic atmosphere (N_2:CO_2:O_2 at ratio of 85:10:5). The mixture was shaken each 20 min to simulate the peristaltic movement of the stomach. In addition to the samples explained above, blank 1 (simulated gastric juice containing spray-dried powder, without *H. pylori*), blank 2 (simulated gastric juice, without *H. pylori*, without spray-dried powder), and control (simulated gastric juice containing *H. pylori*, without spray-dried powder) were also added to the microplates in duplicate. Absorption (A) of the microplates was read at 600 nm by spectrophotometer. Viability of *H. pylori* was calculated according to Eq. 1 [23]:

$$\text{Survivability (\%)} = \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \quad \text{Eq. 1}$$

In calculation, absorption of blank 1 was used for the samples containing the spray-dried powders and absorption of blank 2 was used for the samples containing *Z. multiflora* extract alone.

2.6. Statistical analysis

Data of bacterial survivability was analyzed by SPSS software version 20, and presented as mean ±SD. One-way ANOVA followed by Bonferroni test was used for analysis. Microbial tests were done in duplicate. Differences were significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Anti-Helicobacter activity

As seen in Figure 1, both chitosan and pectin could sucssesfully suppress growht of *H. pylori in vitro*. Although no significant differences were observed among $CE_{0.5}$, $PE_{0.3}$, and P_1, the powder cotaining chitosan and *Z. multiflora* extract had the most inhibitory impact mainly due to the antagonistic effect of chitosan against the bacteria. It is a cationic biopolymer in nature and density of positive charge is increased in acidic environment. Protonation of amine groups in the polymeric backbone at low pH results in ionization of chiosan and its electrostatic interction with negativly charged agents.

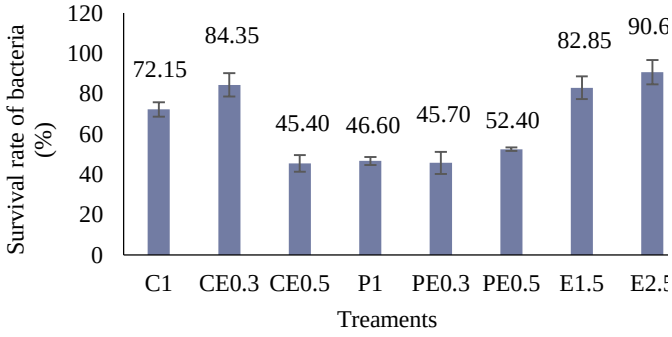
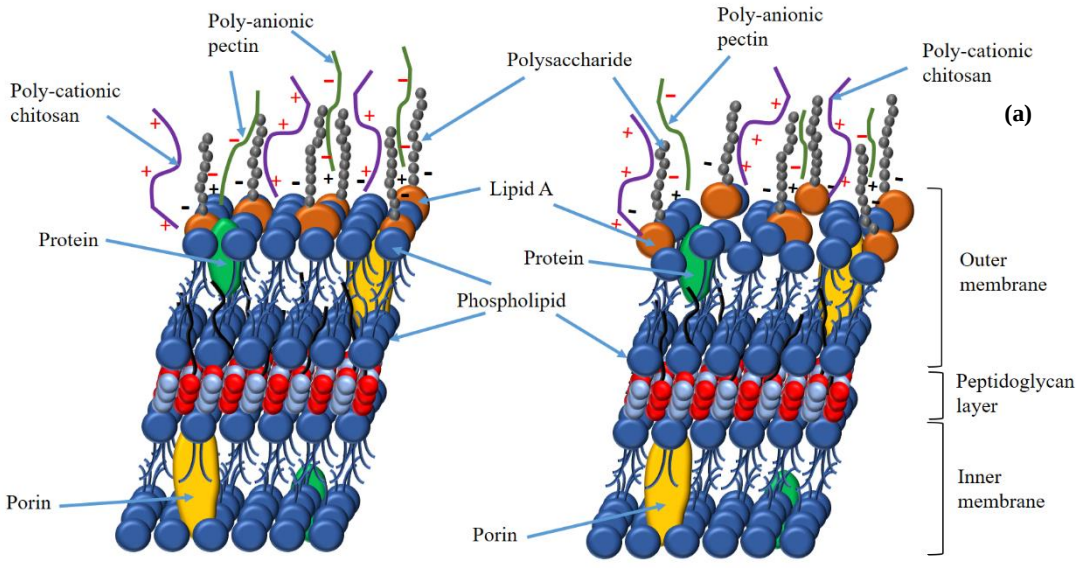


Figure 1. Impact of samples on viability of *Helicobacter pylori* under simulated gastric condition (pH = 3; 37 °C).
C_1: Chitosan 1% w v^{-1}; $CE_{0.3}$: Chitosan 1% w v^{-1} + 0.3 ml *Z. multiflora* extract; $CE_{0.5}$: Chitosan 1% w v^{-1} + 0.5 ml *Z. multiflora* extract, P_1: Pectin 1% w v^{-1}; $PE_{0.3}$: Pectin 1% w v^{-1} + 0.3 ml *Z. multiflora* extract; $PE_{0.5}$: Pectin 1% w v^{-1} + 0.5 ml *Z. multiflora* extract; $E_{1.5}$: 1.5 µl *Z. multiflora* extract; $E_{2.5}$: 2.5 µl *Z. multiflora* extract

Therefore, antibacterial effect of chitosan is driven by its electrostatic interaction with negative charge of bacterial outer membrane, which mainly composed of lipopolysaccharid (Figure 2a). As a result, the bacterial integrity is changed and cells' viabiliy is affected due to the changes in their permeability and loss of vital molecules [24]. In addition, biopolymers may interact with the nutrients in the environment and made them unavailable for microorganisms. Antimicrobial activity of chitosan is exacerbated by increasing its charge density. Indeed, chitosan is derived from chitin in which acetyl groups are attached to amine residues. During the process of chitosan synthesis, acetyl groups are removed and antimicrobial activity of chitosan is achieved thanks to the amine resudies available to further interactions. Importantly, we used chitosan with deacetylation degree over 75% in the current work. Findings of our microbial analysis were approved by the results of zeta potential (Figure 3), through which the highest charge density was observed for chitosan.

Several studies have reported antibacterial acrivity of pectin. In study of Daoud et al., antagonistic effect of high methoxy pectin against *H. pylori* was investigated *in vitro*. Although, anti-Helicobacter activity of pectin was lower than ampicillin in their study, but a lower minimum inhibitory concentration (MIC) was achieved for pectin than root extract of *Terminalia macroptera*, that is a natural source used for control and treatment of *H. pylori* (MIC 0.162 μg μl^{-1} for pectin vs. 200 μg μl^{-1} for *T. macroptera* root extract) [21]. It was assumed that ion exchange ability of pectin was responsible for its antimicrobial activity [21,25]. The uronic acids such as galactoronic acids in pectin structure can exchange ions in the environment [26] (Figure 2a). *H. pylori* is a Gram-negative bacterium composed of a thin layer of peptidoglycan. It is coverd by outer membrane made of lipoposaccharide containing a lot of phosphorus groups in the phospholipid layer. The outer membrane is strengthern by the cations of magnesium and calcium as a brige between the phosphorus groups. Cationic antibiotics and chelating agents such as EDTA can disturb the bacterial cell integrity by interfering in normal function of the cationic poles [27]. Anionic carboxyls in pectin structure can interact with the cationic cross-linkers in the bacterial outer membrane resulted in reduced cell viability. Negative zeta potential of the all pectin-containing samples (Figure 3) strengths this hypothesis. Moreover, anti-adhesive role has been reported for pectin, which can hinder *H. pylori* attachment to the gastric epithlium [27]. Although, the current study was conducted in the laboratry and the latest charactersitic of pectin was not involved in the bacterial repression of our work. Nonetheless, the underlying antibacterial mechanism of pectin is not well understood, so that its antagonistic effect was only observed against *H. pylori* and no significant inhibition was reported against *Escherichia coli* and *Klebsiella pneumoniea* in the study of Daoud et al. [21].

With respect to the synergism by addition of *Z. multiflora* extract to the polymers in suppresion of *H. pylori*, role of thymole and carvacrol as the main phenol compounds in the extract (calculated as 13.86% and 40.13%, respectively) in disruption of the bacterial cells' integrity is of importance. In this regard, microbial cytoplasm is depleted of ATP in the presence of phenolic compounds, which leads to cell death (Figure 2b) [8,9]. Due to a relatively low solubility of chitosan and pectin in aqueous media, it was expected to find the highest anti-Helicobacter activity for pure extract ($E_{1.5}$ and $E_{2.5}$), but they were not effective enough likely because of their low concentration. On the other hand, addition of *Z. multiflora* extract at high concentration to the formula was not possible due to their adhesion to the spray dryer. Therefore, other approaches such as freeze drying may be useful for this purpose to facilitate the use of *Z. multiflora* extract at higher concentrations.



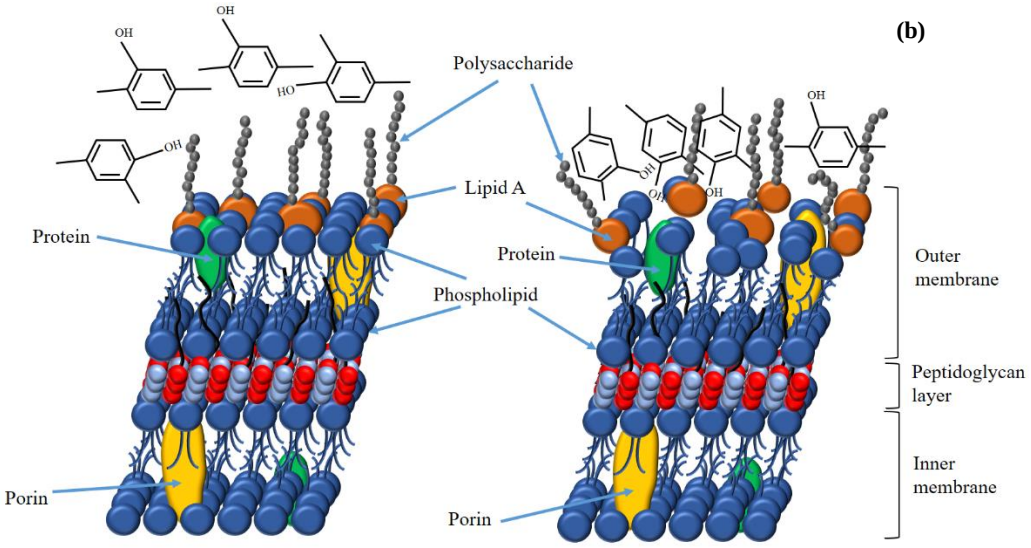
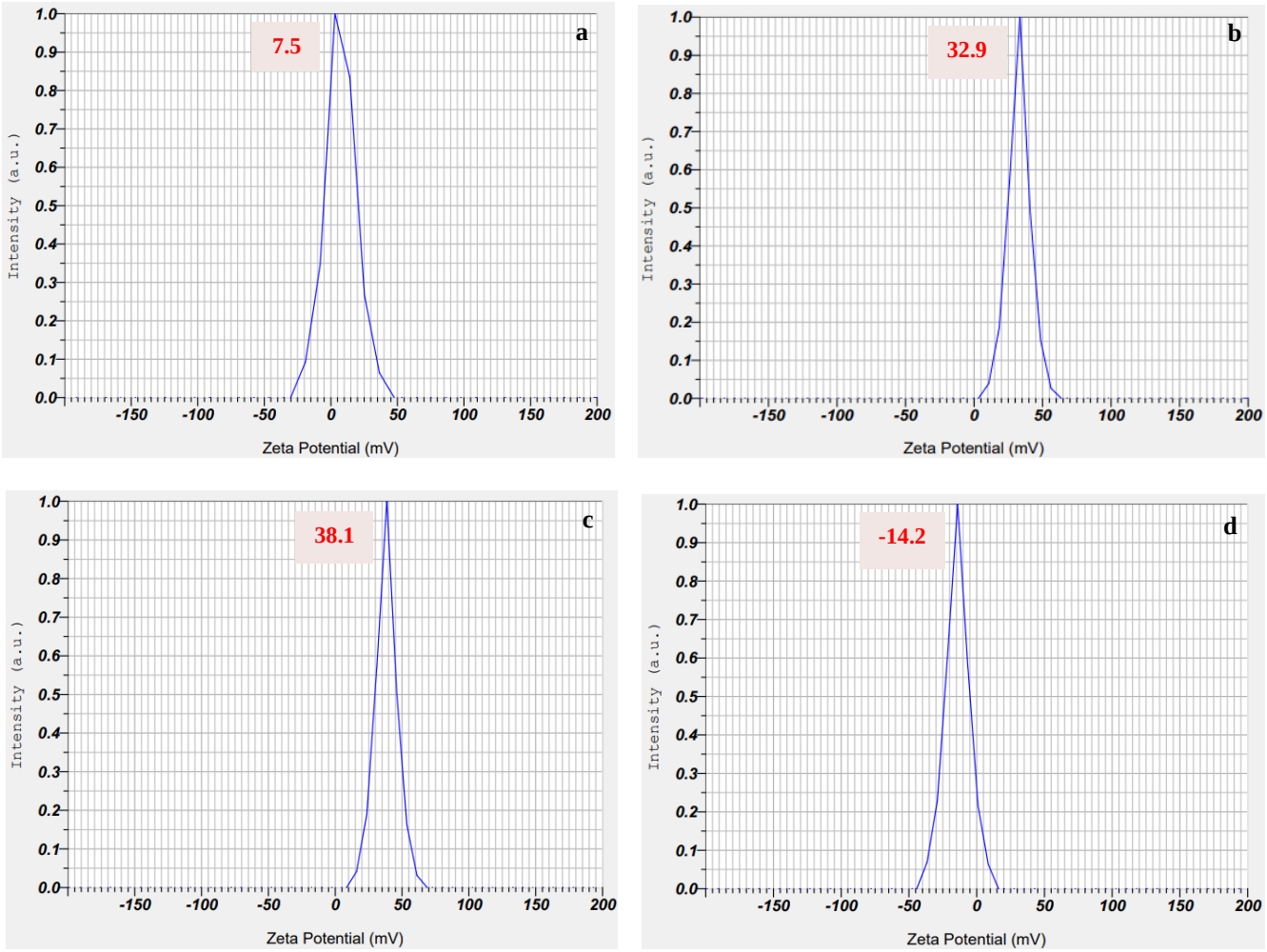


Figure 2. Shcematic of antimicobial activity of a) chitosan and pectin, and b) phenolic compounds againts Gram-negative bacteria. In Figure 2a, the red + and - are charge density of chitosan and pectin, respectively, and the black + and - are charge density of bacterial outer membrane.



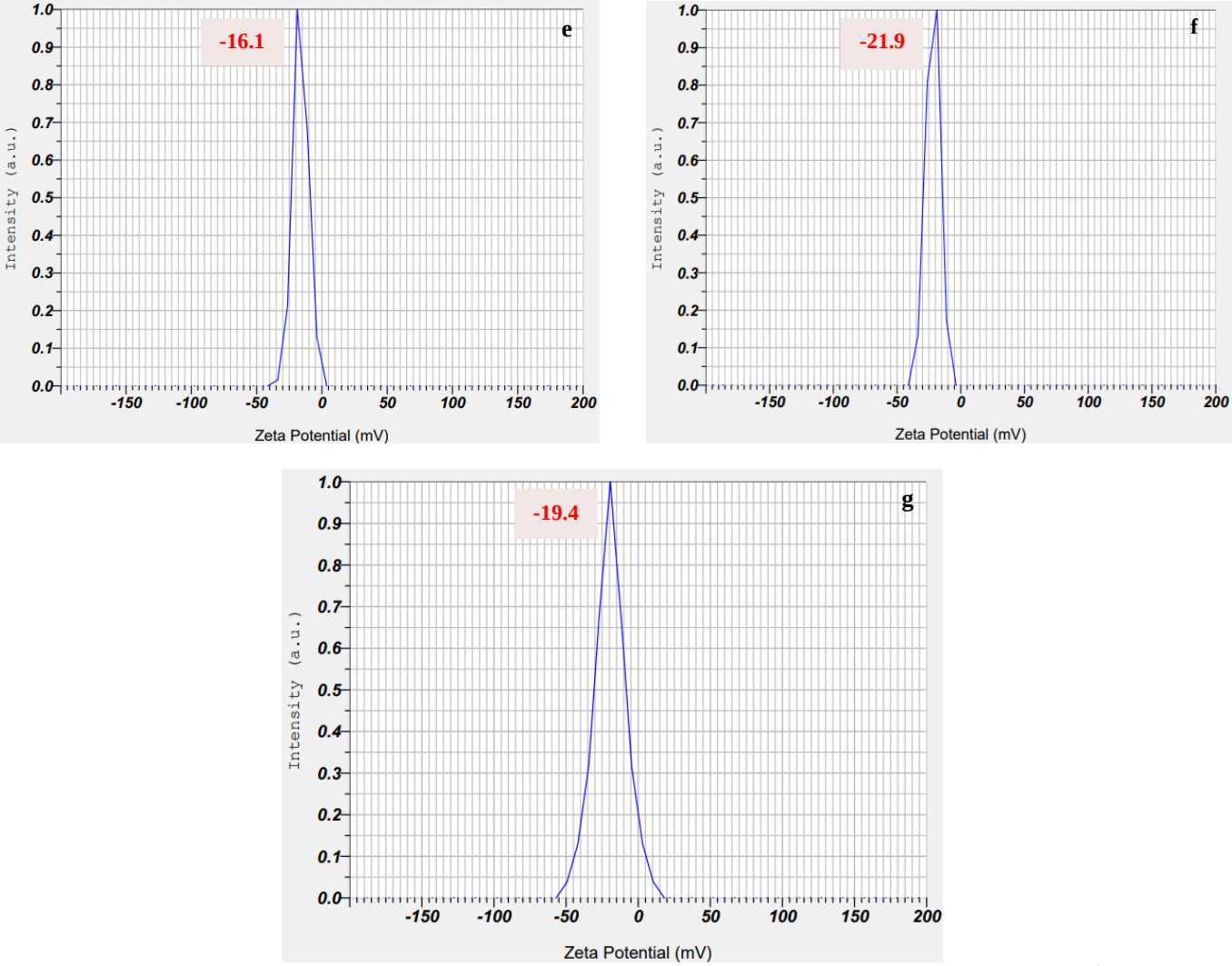


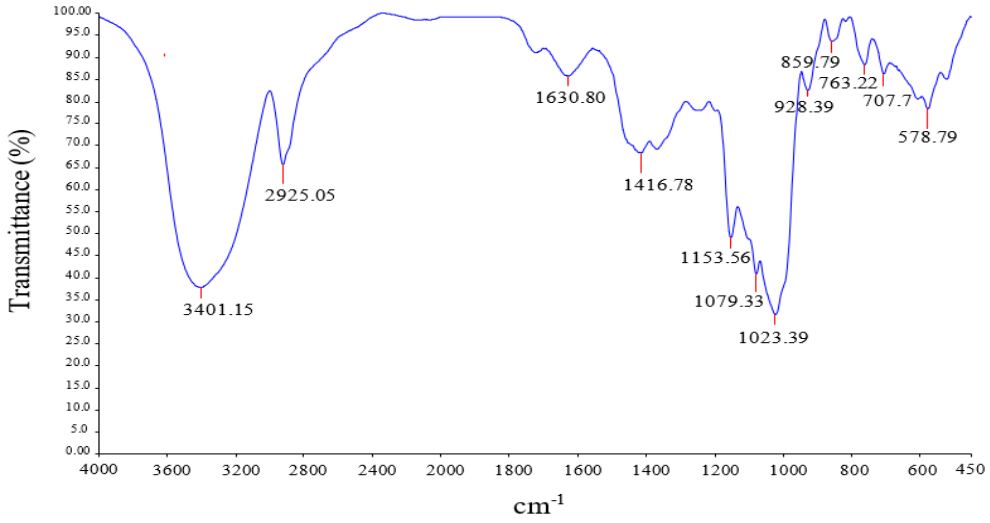
Figure 3. Zeta potentials of a) C_1, b) $CE_{0.3}$, c) $CE_{0.5}$, d) P_1, e) $PE_{0.3}$, f) $PE_{0.5}$, and g) extract. C_1: Chitosan 1% w v^{-1}; $CE_{0.3}$: Chitosan 1% w v^{-1} + 0.3 ml *Z. multiflora* extract; $CE_{0.5}$: Chitosan 1% w v^{-1} + 0.5 ml *Z. multiflora* extract; P_1: Pectin 1% w v^{-1}; $PE_{0.3}$: Pectin 1% w v^{-1} + 0.3 ml *Z. multiflora* extract; $PE_{0.5}$: Pectin 1% w v^{-1} + 0.5 ml *Z. multiflora* extract

3.2. Chemical interactions

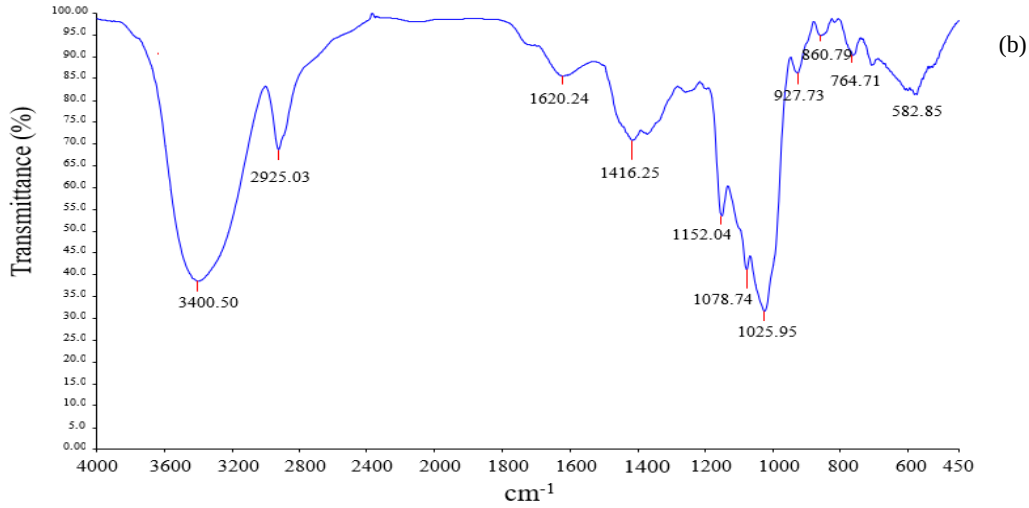
FT-IR analysis is a fast and simple method for detection of functional groups and their chemical interaction within the structures based on absorbance or transmittance data [28,29]. As seen in Figure 4a, the dip appeared at 3400 cm^{-1} was related to stretching O-H which prominently exists in pectin [30]. Moreover, this wave number can also be related to N-H group in the amidated pectin [31]. Although, the amidation degree of pectin was low and nonsignificant change was observed in the spectrum appeared for PE sample (Figure 4b), followed by hydrogen interaction between amidated pectin and phenolic compounds in the extract. Presence of alkane groups of C-H and C-C in both P_1 and PE samples is confirmed by the dips appeared at 2925 and 580-800 cm^{-1}, respectively [29]. Carboxyl, as the main functional group of pectin, is detected by the dips at 1620-1630 cm^{-1} [30]. The small dips observed at 1000-1200 cm^{-1} are likely related to C-N in amidated pectin [29]. Importantly, no significant change is observed after addition of *Z. multiflora* extract to pectin. It indicates that the extract was physically entrapped by pectin chains or adsorbed on surface of polymeric chains without any covalent bond. Although, the small shift from 1630 cm^{-1} in P_1 to 1620 cm^{-1} in $PE_{0.5}$ was probably due to the presence of conjugated C=C in phenolic compounds of *Z. multiflora* [14], which poses a similar transmittance manner with carboxyl groups of pectin and their simultaneous inclusion in the mixture resulted in a little change in the graph (Figures 4a and 4b). Similar results were observed in the study of Salehi et al. about addition of thyme EO to pectin-based nano-emulsion for therapeutic purposes in treatment of cancer cells. They observed no significant change in FTIR spectra by addition of EO [29].

Similar to pectin, functional groups of O-H, C-H, C=O, and C-N are also observed in chitosan. Presence of O-H and N-H in chitosan is indicated by the dip appeared at 3434 cm^{-1} (Figure 4C), which shifted to 3401 cm^{-1} in $CE_{0.5}$ (Figure 4d). The change might be due to hydrogen interaction between phenolic O-H in the extract and nitrogen atoms in chitosan. Moreover, the new dip at 1024 cm^{-1} (Figure 4d) is likely related to stretching C-O after addition of *Z. multiflora* extract to chitosan [32]. Abundance of phenol compounds in the extract increased the density of C-O groups, which further appeared by a new dip in $CE_{0.5}$ sample. As mentioned for PE sample, the most possible chemical change in the CE sample was driven by hydrogen interactions between O-H groups of *Z. multiflora* extract and nitrogen groups of chitosan. It also indicates physical inclusion of *Z. multiflora* extract in chitosan without strong chemical bond.

(a)



(b)



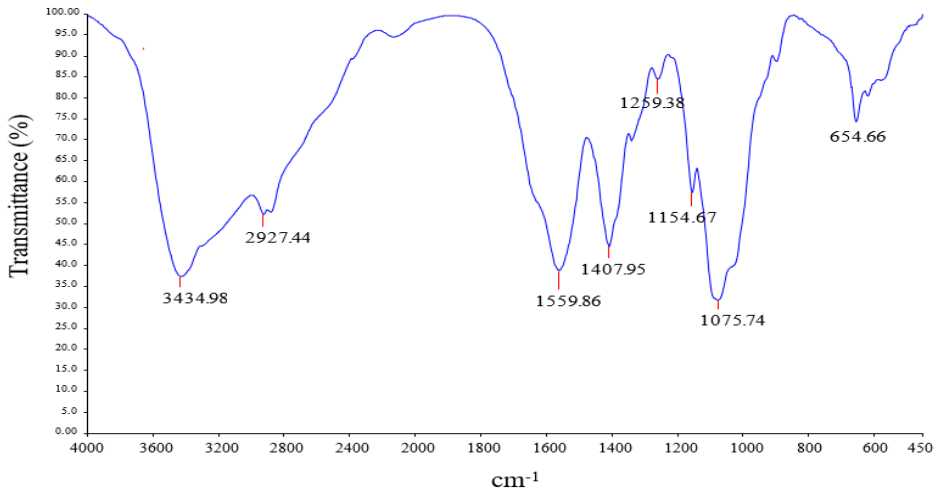


Figure 4. FT-IR spectra of a) P_1, b) $PE_{0.5}$, c) C_1, and d) $CE_{0.5}$. P_1: Pectin 1% w v^{-1}; $PE_{0.5}$: Pectin 1% w v^{-1} + 0.5 ml *Z. multiflora* extract; C_1: Chitosan 1% w v^{-1}; $CE_{0.5}$: Chitosan 1% w v^{-1} + 0.5 ml *Z. multiflora* extract

3.3. Morphology

SEM graphs of $CE_{0.5}$, $PE_{0.3}$, and P_1 samples, which showed the most anti-Helicobacter activity *in vitro*, are presented in Figure 5. As seen in the figure, the particles had spherical and smooth shape. Small depression is observed on surface of some particles that is probably due to surface tension driven by the extract and water evaporation during spray drying [30]. It is worth noting that size of the particles was calculated as 4.36 ±2.7 µm for $CE_{0.5}$, 3.71 ±2.3 µm for $PE_{0.3}$, and 5.7 ±2.96 µm for P_1 samples.

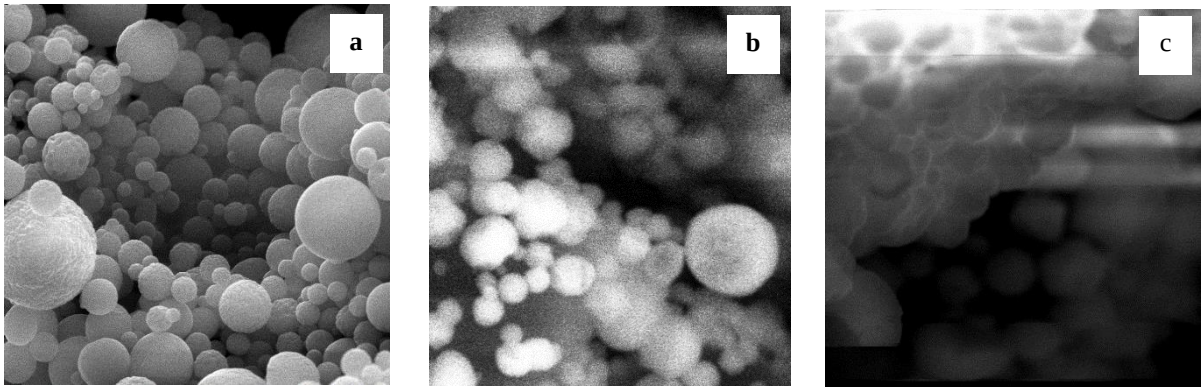


Figure 5. SEM graphs of a) $CE_{0.5}$, b) $PE_{0.3}$, and c) P_1, with ×5000 magnification; $CE_{0.5}$: Chitosan 1% w v^{-1} + 0.5 ml *Z. multiflora* extract; $PE_{0.3}$: Pectin 1% w v^{-1} + 0.3 ml *Z. multiflora* extract; P_1: Pectin 1% w v^{-1}

4. Conclusion

It this study, we evaluated the anti-Helicobacter activity of *Z. multiflora* extract containing micro-capsules prepared by chitosan and pectin. Interestingly, our samples could successfully decrease survivability of *H. pylori* under simulated gastric condition. Both chitosan- and pectin-based micro-particles showed similar impact in this regard. It is expected that the anti-Helicobacter activity was driven by phenolic compounds in *Z. multiflora* extract, electrostatic interaction of chitosan with the negatively charged surface of the bacteria, and ion exchange ability of pectin. Weak chemical interactions and physical entrapment of *Z. multiflora* extract in the biopolymers were of interest. Given the significant synergistic effect of *Z. multiflora* extract with chitosan and pectin in *H. pylori* suppression, further *in vivo* trials are recommended. Furthermore, development of trials by using the introduced formula as alternative or complementary treatment for the common antibiotics used in *H. pylori* eradication is suggested.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

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ساخت، بررسی خصوصیات، و ارزیابی فعالیت ضدهلیکوباکتریایی ریزکپسول‌های کیتوزان و پکتین حاوی عصاره آویشن شیرازی در شرایط آزمایشگاهی

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چکیده

سابقه و هدف: عفونت *هلیکوباکتر پیلوری* از جمله بیماری‌های شایع در جهان است. رایج‌ترین روش درمان برای از بین بردن این بیماری استفاده از آنتی‌بیوتیک‌ها است که در برخی از بیماران چندان اثربخش نبوده و منجر به مقاومت به آنتی‌بیوتیک در آنها می‌شود. نظر به اثرات نامطلوب برخاسته از مصرف آنتی‌بیوتیک‌ها که در مطالعات بالینی به آن اشاره شده است. در این مطالعه ضمن تهیه ریزکپسول‌های بر پایه کیتوزان و پکتین حاوی عصاره آویشن شیرازی، اثر سرکوب‌کنندگی آنها علیه *هلیکوباکتر پیلوری* در شرایط آزمایشگاهی بررسی شد.

مواد و روش‌ها: ریزکپسول‌های کیتوزان و پکتین به طور مجزا و در ترکیب با عصاره آویشن شیرازی با دستگاه خشک‌کن پاششی تهیه شدند. ویژگی نمونه‌های تهیه شده با دستگاه طیف‌سنج مادون قرمز تبدیل فوریه، دستگاه اندازه‌گیری پتانسیل زتا، و میکروسکوپ الکترونی روبشی مورد بررسی قرار گرفت. برای ارزیابی اثر ضدهلیکوباکتریایی نمونه‌ها، محیط شبیه‌سازی شده معده در آزمایشگاه تهیه شد.

یافته‌ها و نتیجه‌گیری: نمونه‌های کیتوزان ۱ درصد وزنی-حجمی حاوی ۰/۵ میلی‌لیتر عصاره آویشن شیرازی، پکتین ۱ درصد وزنی-حجمی حاوی ۰/۳ میلی‌لیتر عصاره آویشن شیرازی، و پکتین ۱ درصد وزنی-حجمی به طور معنی داری جمعیت *هلیکوباکتر پیلوری* تلقیح شده در محیط شبیه‌سازی معده را کاهش دادند. کمترین نرخ زنده‌مانی *هلیکوباکتر پیلوری* ۴۵/۴ درصد بود که به محیط تلقیح شده با نمونه کیتوزان ۱ درصد وزنی-حجمی حاوی ۰/۵ میلی‌لیتر عصاره آویشن شیرازی تعلق داشت. نمونه‌های پکتین ۱ درصد وزنی-حجمی حاوی ۰/۳ میلی‌لیتر عصاره آویشن شیرازی، و پکتین ۱ درصد وزنی-حجمی به ترتیب هر کدام ۴۵/۷ درصد و ۴۶/۶ درصد زنده‌مانی باکتری را در محیط شبیه‌سازی معده منجر شدند. بار الکتریکی کیتوزان و پکتین و همچنین ترکیبات فنولی عصاره آویشن شیرازی باعث تخریب دیواره سلول باکتریایی گردیدند. طبق نتایج طیف‌سنجی مادون قرمز تبدیل فوریه، عصاره آویشن شیرازی به طور فیزیکی درون لایه‌های کیتوزان و پکتین محصور بود که این امر باعث اثربخشی بیشتر آن شد. با توجه به اثر ضدهلیکوباکتریایی نمونه‌های مطالعه حاضر، این ترکیبات می‌توانند به عنوان تیمار کمکی همراه با آنتی-بیوتیک‌ها در درمان عفونت *هلیکوباکتر پیلوری* مورد استفاده قرار گیرند. اگرچه به مطالعات درون‌تن برای تایید یافته‌های این مطالعه نیاز است.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- *هلیکوباکتر پیلوری*
- پکتین
- محیط شبیه سازی شده معده
- آویشن شیرازی

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- **بهروز جنت**

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