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Original Article

In vitro anti-*Helicobacter pylori* activity of aqueous extract from Persian Oak testa

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ABSTRACT

Objective: Growing problem of antibiotic resistance in *Helicobacter pylori*, as a common cause of chronic gastritis and even stomach cancer, demands searching for novel candidates of herbal sources. This study is aimed at assessing the antimicrobial activity of aqueous extract obtained from *Quercus brantii* var. *persica* seed coat (Testa) on *H. pylori* isolated from gastric biopsy specimens.
Methods: Such specimens were collected from 100 patients presenting with endoscopic gastroduodenal findings. Testa extracts were prepared from Persian Oak forests in the province of Kohgiluyeh and Boyer-Ahmad, IRAN. *H. pylori* isolates were obtained by a series of standard bacteriology tests and cell culture, then were confirmed by PCR. The activity of testa extracts towards 25 *H. pylori* isolates was assessed by well diffusion method, microdilution assay, and a disk diffusion assay *in vitro*. Results were analyzed statistically by one-way ANOVA analysis.
Results: Aqueous extract of testa demonstrated an antimicrobial activity with zone diameters of inhibition ranged from 0 mm to 40 mm. Its inhibitory activity increased simultaneously with increasing extract concentration. The lowest MIC and MBC were both recorded as 2 μg/mL. Anti-*H. pylori* activity of testa extract was approximately close to tetracycline and metronidazole and less than amoxicillin. A potent extract of testa possessed significant inhibitory activity ($P < 0.05$).
Conclusion: Testa extract is suggested as a natural therapeutic source against the gastric *H. pylori* infection. However, evaluating the *in vivo* activity of this extract is necessary too.

1. Introduction

Helicobacter pylori is a Gram-negative bacteria, which causes many gastric diseases, and infects approximately 50% of the world's population (Wang, Meng, Wang & Qiao, 2014). *H. pylori* commonly known as a cause of chronic gastritis, if left untreated or unsuccessfully treated, can lead to ulcers, and even stomach cancer (Wang et al., 2014). Progress on antibiotic resistance in *H. pylori* to common medicines like clarithromycin, metronidazole, and amoxicillin leads to deactivate the current antibiotic therapies (Ben Mansour et al., 2010). Hitherto, several international guidelines are presented for treatment of *H. pylori* infected patients based on the triple therapy as the first-line treatment; These treatments include administering a proton pump inhibitor (PPI), clarithromycin, and amoxicillin for 1 – 2 weeks (Chey & Wong, 2007; Malfertheiner et al., 2007). However, the eradication therapy of *H. pylori* infections via this therapeutic system has been successful at less than 80% of cases, principally due to increase in clarithromycin resistance (Ayala, Escobedo-Hinojosa, de la Cruz-Herrera & Romero, 2014). As a result, other regimens (second-line therapies) have been proposed (Ayala et al., 2014). Second-line therapy usually consists of a PPI along with two or three antibiotics (amoxicillin, clarithromycin, metronidazole, and tetracycline). However, this treatment still fails to eradicate *H. pylori* in 20%–30% of patients (Fischbach & Evans, 2007; Foroumadi et al., 2008). Various studies have shown the emergence of resistance to common antibiotics, the side-effect rates of tetracycline and metronidazole, and high cost of combination therapy; Thus, these lead to reducing patient compliance and patient unsteadiness (Li, Xu, Zhang, Liu & Tan, 2005; O'Gara, Hill & Maslin, 2000; Sherif et al., 2004). Accordingly, the search for discovering new and effective anti-*H. pylori* drugs is very important, and plants are a safe source of such new logical compounds (Castillo-Juareza et al., 2009).

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There are a large number of scientific publications worldwide describing the anti-*H. pylori* activity of herbal products. In 1991, a study on 13 Malagasy medicinal plants showed a significant anti-*H. pylori* effect of this plant (Cassel-Beraud, Le Jan, Mouden, Andriantsoa & Andriantsiferana, 1991). Ever since then, many researches about the medicinal plants from various specific areas or countries have been conducted; These studies include the anti-*H. pylori* activity of Chinese (Higuchi et al., 1999), Mexican (Castillo-Juareza et al., 2009), Iranian (Hajimahmoodi et al., 2011; Nariman, Eftekhar, Habibi & Falsafi, 2004), African (Filomena Garro et al., 2015) and Greek (Stamatis et al., 2003) herbal medicines.

Persian Oak (*Q. brantii* var. *persica*) is a predominant plant genus in northern and central Iran comprised of many species. Persian Oak, covering more than 50% of the Zagros forest area, is the most important tree species of Zagros in Iran. This plant is used for treatment of gastric disorders by Native Iranians in Zagros region (Chahar Mahal VA Bakhtiyari, Ilam and Kohgiluyeh VA Buyer Ahmad Provinces) and southwestern Iran (Heydari, Poorbabaei, Rostami & Ostad, 2013). Compounds extracted from fruit and leaves of several *Quercus* spp. have shown the antioxidant activity, and these are applied as traditional drugs in the treatment of diseases like cancer (Rocha-Guzman, Gonzalez-Laredo & Gallegos-Infante, 2007). Some studies showed that tannin (polyphenolic) compounds of *Quercus spp*. can cause the gastroprotective effects (Khennouf et al., 2003; Rocha-Guzman et al., 2009). *Quercus* spp. fruit (acorn) has outer and inner layers including cupule, pericarp (fruit wall), seed coat (testa), cotyledons (2), plumule, and radicle, respectively. Seed coat or testa of *Q. brantii* var. *persica* is known locally as *Jaft*. Although testa is usually yellow, its color changes to brown after exposure to light due to oxidation. Aim of this study was to evaluate the antimicrobial activity of aqueous extract obtained from Persian Oak seed coat (testa) on *H. pylori* isolates obtained from biopsy specimens; Our findings help to identify potential low-cost sources of therapeutic medicinal plants.

2. Materials and methods

2.1. Collection and identification of plant materials

Testa layers (seed coat) of *Q. brantii* var. *persica* were collected from Persian Oak forests in the province of Kohgiluyeh and Boyer-Ahmad located in southwest of Iran on March 2016. Its geographical coordinates is between latitudes 30–9° to 31–27°N and longitudes 49–55° to 51–42°E.

Identification and taxonomic classification of plant materials were done in the Botanical Research Institute of Yasuj University of Medical Sciences. A test specimen (QBMP 3/21/2016) was sent to the Herbal Medicinal Research Center, Yasuj University of Medical Sciences.

2.1.1. A preliminary study on prevalence of H. pylori gastric infection

Pathology reports of patients obtained from clinical pathology labs were initially reviewed to estimate the prevalence of gastroduodenal complications associated with *H. pylori* infection in the city of Yasuj, the capital of Kohgiluyeh and Boyer-Ahmad in Iran.

2.2. Preparation of plant extracts

Firstly, the fruits of *Q. brantii* var. *persica* were washed and dried, and then testa layer or seed coat of *Q. brantii* var. *persica* was collected. A fine powder (20 mesh) was then obtained from testa specimens using a Retsch Ultra Centrifugal Mill (Haan, Germany). Aqueous extract was prepared by dissolving 200 g powdered material with 1440 mL distilled water (DW) by the maceration method (Sarker, Latif & Gray, 2005) for 24 h. Aqueous extract was mixed and combined with 560 mL solvent (DW decoction), filtered by Whatman NO.1 filter paper, and then concentrated and dried by using a rotary evaporator (Heidolph model 4000; Germany). Dried extract was stored at 4 °C for further studies. The concentration of the bulk aqueous extract was 10^5 µg/mL.

2.3. Bacterial isolates and culture conditions

A total of 25 strains of *H. pylori* isolated from antral mucosal biopsies of patients with endoscopic gastroduodenal findings were used; Gastric biopsy specimens were collected from 100 patients (age range from 13 to 83; 42 men and 58 women) referred to the Gastroenterology Unit of Yasuj Hospital in the province of Kohgiluyeh and Boyer-Ahmad, Iran. *H. pylori* strains were cultured by inoculating biopsies on *Campylobacter* selective agar (CSA) containing 4% blood base agar No. 2 (Oxoid), defibrinated horse blood (Oxoid), and one vial of Skirrow's supplement (Oxoid) containing 2.5 mg Trimethoprim, 5.0 mg Vancomycin, and 1250 IU polymyxin B (Sharifi et al., 2009; Stamatis et al., 2003). Primary cultures were incubated at 37 °C with 10% CO_2 in a CO_2 incubator (Plymouth, USA) for up to 10 d, observing daily for growth. To attain the pure cultures, a single colony was picked and subcultured onto CSA for 4 – 5 d (Sharifi et al., 2009; Stamatis et al., 2003). *H. pylori* strains were identified based on the colony appearance, Gram staining, and positive reactions in differential biochemical tests (catalase, urease, and so on). Strains were stored at −70 °C in brain heart infusion broth (BHI, Difco, MD, USA) containing 40% glycerol (Sharifi et al., 2009).

2.4. DNA extraction

DNA was extracted by using a 502 MagCore® Nucleic Acid Extraction Kit (Taiwan) according to the manufacturer's instruction. Standard strain of *E. coli* ATCC 12651, code: CBD 02330 (LTA company, Italy) was used as negative control; And standard strain of *H. pylori* with code: GC439 (Pasteur Institute of Iran) was used as positive control.

2.5. PCR amplification of H. pylori 16S rRNA gene

Presence of specific *H. pylori* 16S rRNA sequence was confirmed by using an automated AmpliSens® *Helicobacter pylori*-FRT PCR Kit (Central Research Institute for Epidemiology; Moscow, Russia) according to the manufacturer's instruction. This test qualitatively detects *H. pylori* DNA in clinical material (biopsy specimens of gastric mucosa) by using real-time hybridization-fluorescence. Total reaction volume is 25 µL and DNA sample volume is 10 µL (10 ng/µL). This was carried out based on the presence of ureC fragment (glmM gene) and by using specific 16S rRNA primers and 23S rRNA primers according to the program described by the manufacturer. The program of the real-time amplification is according to manufacturer's manual and guidelines (Guidelines by Federal Budget Institute of Science, 2014; Handbook by Federal Budget Institute of Science, 2008). A temperature profile was created on the device as follows (Table 1).

Result interpretation was done using a software by crossing the fluorescence curve with the threshold line. Principle of interpretation was given in Table 2. FAM/Green fluorescence channel indicates Internal Control DNA and JOE/Yellow/HEX fluorescence channel indicates *H. pylori* DNA (Guidelines by Federal Budget Institute of Science, 2014; Handbook by Federal Budget Institute of Science, 2008).

2.6. Screening of crude extract for anti-H. pylori activity

Antimicrobial activities of aqueous extract obtained from testa layer were evaluated by the agar well diffusion method

Table 1
AmpliSens-1 amplification program of *H. pylori* 16S rRNA gene.

Steps	Rotor-type instruments			Plate-type instruments		
	Temperature/°C	Time	Cycles	Temperature/°C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling	95	5 s		95	5 s	
	60	25 s			25 s	
		fluorescent acquiring	45		fluorescent acquiring	45
	72	10 s		72	10	

Note: Fluorescent signal is detected in FAM and JOE fluorophores channels. Fluorescence channel sensitivity was regulated according to product information and guidelines (Guidelines by Federal Budget Institute of Science, 2014).

Table 2
Interpretation of amplification's result.

Ct value in channel		Interpretation
FAM/Green	JOE/Yellow/HEX	
Ct value is defined	Positive (< 20)*	*H. pylori* DNA is detected
Positive (< 20)	Negative (> 35) or undefined*	*H. pylori* DNA is not detected
Negative (> 35) or undefined	Negative (20 < Ct value < 35) or Undefined*	Invalid result
≤ boundary value	> boundary value or absent	Negative control
> boundary value or absent	≤ boundary value	Positive control + *H. pylori*

Note: FAM/Green fluorescence channel indicates Internal Control DNA and JOE/Yellow/HEX fluorescence channel indicates *H. pylori* DNA. Results were interpreted by crossing (or non-crossing) fluorescence curve with threshold line located at defined level concerning to the presence (or absence) of a threshold cycle value (*Ct* value) of DNA sample in corresponding column of results grid. The presence of *H. pylori* DNA is positive if *Ct* value in FAM channel is defined and in JOE channel is ≤ 20 mm. While *Ct* value in FAM channel is < 20 mm and in JOE channel is ≥ 35 mm, presence of *H. pylori* DNA is negative.

* Boundary Ct values were recorded between 10.19 and 37.58.

(Boyanova et al., 2005), and disk diffusion assays as previously reported (Castillo-Juareza et al., 2009; Ndip, Malange, Mbullaha, Lumab & Malongue, 2007). Briefly, *H. pylori* inocula prepared at McFarland's turbidity standard 2 was swabbed on Mueller Hinton agar (CM0337, Oxoid, England). Inoculum obtained from bacterial subcultures was evenly spread on the plate by sterile swab and allowed to dry for 5–8 min. Filter paper disks (6 mm diameter) were smeared with 5 mg of aqueous extract (= 50 µL) and placed onto the surface of the inoculated agar. Antibiotic disks containing 25 µg of amoxicillin (Amx), 50 µg of metronidazole (Mtz), 30 µg of tetracycline (Tet) as positive controls, and distilled water (DW) soaked disk (negative control) were dispensed onto the surface of agar. Plates containing the mentioned materials were incubated at 37 °C under microaerophilic conditions and checked after 3 – 5 d. All tests were carried out in triplicate and the antimicrobial activity was illustrated on the base of the mean diameter of the inhibition zone around the disks.

Results of the disk diffusion method were used to detect potentially active extracts and for minimum inhibitory concentration (MIC) determination using the broth microdilution method. Eventually, the results of the disk diffusion method were compared to the results of broth microdilution method.

2.7. Broth microdilution method

Microdilution method was applied to determine the MICs of the plant extracts using microtitration plates (96 wells) as previously described by Liu, Li, Shang, Zhang and Tan (2016). First, 180 µL of the broth was added into each well on the first row (row A) of microtitration plates and 100 µL was added to the rest of the wells at the second row downwards. Then, 20 µL of the aqueous extract was added into each well on the first row, starting with the positive control (tetracycline), followed by the negative control [20% Dimethyl sulfoxide (DMSO) used to dissolve the plant extracts] and the aqueous extract in the rest of the wells on row A. A twice serial dilution was performed by mixing the contents in the first row and then transferring 100 µL to the second well at the same column. This trend went towards the last well of the same column and the last 100 µL at the last well was thrown away. Then 100 µL of bacterial (*H. pylori* strains) suspensions (1×10^6 CFU/mL) was added.

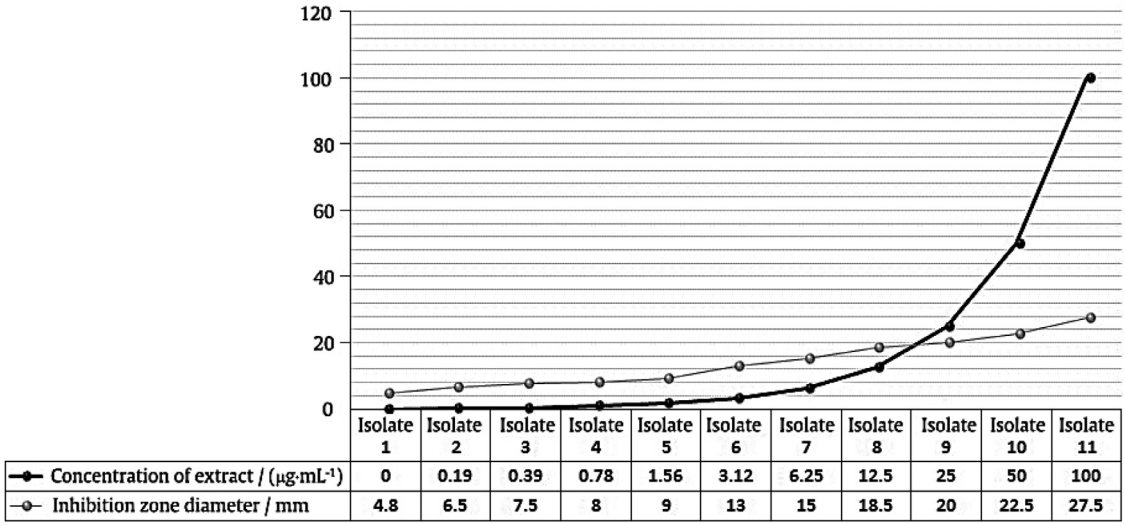
Results were observed after 72 h incubation at 37 °C, followed by the addition of 40 µL of a 0.2% Iodo Nitro Tetrazolium (INT) solution after a further incubation of 4 h at 37 °C.

2.8. Determination of minimum inhibitory concentration (MIC)

The MIC of active extract was assessed by the microdilution method. MICs of all the extracts were determined by diluting the extract in different concentrations (5.000 to 0.150 mg/mL). These concentrations of aqueous extracts and controls were prepared in serial twofold dilutions using Muellar Hinton Broth (MHM). Tubes were incubated at 37 °C for 72 h, and then checked for turbidity indicating the microorganism growth. Lowest concentration of the extract that inhibits the growth of the microorganism based on the lack of visual turbidity (relevant to the negative growth control) was determined as the minimum inhibitory concentration. The turbidity was assessed by visual comparison to the 0.5 McFarland. About 100 µL of bacterial (*H. pylori* strains) suspensions (1×10^6 CFU/mL) was used for this step and the following processes as well.

2.9. Determination of minimum bactericidal concentration (MBC)

Bactericidal activity of the aqueous extract was examined by counting the number of bacteria in the primary microorganism suspension using the surface plate method. After determining the MIC, the number of bacteria was counted in each of the broth tubes showing no visible turbidity after overnight incubation, and was compared with the number of bacteria in the primary microorganism suspension. According to NCCLS (1997), the lowest concentration of the extract that allowed to survive less than 0.1%



	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10	Isolate 11
Concentration of extract / (μg·mL^{-1})	0	0.19	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100
Inhibition zone diameter / mm	4.8	6.5	7.5	8	9	13	15	18.5	20	22.5	27.5

Fig. 1. Inhibitory concentration and inhibition zone diameter of antimicrobial extract of testa layer of *Q. brantii* var. *persica* against *H. pylori* isolates by agar well diffusion method. The lowest concentration of testa extract that prevents visible growth of *H. pylori* was 1.56 μg/mL based on the inhibition zone diameter.

of the primary inoculum was considered as the minimum bactericidal concentration.

3. Results

3.1. Prevalence of H. pylori gastric infection

In preliminary study, the prevalence rate of *H. pylori* gastric infection was estimated 68% of all patients referring to the clinical pathology labs in the city of Yasuj. Moreover, the prevalence of *H. pylori* gastric infection was 71.42% among men and 65.5% among women. Prevalence was respectively highest among those aged 41–52 years (81.8%) and then those aged 30–41 years. Main research study was carried out on 100 patients who were randomly selected among the most prevalent of all *H. pylori*-infected patients in both sexes.

Of 100 patients, 31 (31%) were infected with *H. pylori* based on the culture method, subcultured *H. pylori* colonies onto CSA, and positive reactions in differential biochemical tests (Sharifi et al., 2009; Stamatis et al., 2003). Specific *H. pylori* 16S rRNA sequence was found in 25 (80.65%) out of 31 putative *H. pylori* isolates. Antimicrobial activity of aqueous extracts against these 25 *H. pylori* isolates was studied.

3.2. Antimicrobial activity of extracts

In vitro evaluating of anti-*H. pylori* activity of aqueous extracts obtained from *Q. brantii* var. *persica* using agar well diffusion method demonstrated that the lowest effective concentration of the extracts was 1.56 μg/mL (Fig 1). Although *P* value was not statistically significant ($P > 0.05$) at this concentration (1.56 μg/mL), it was significant ($P < 0.05$) in higher concentrations of the extracts (>1.56 μg/mL).

Aqueous extract showed significant inhibitory effect on *H. pylori* isolates in assessment of the anti-*H. pylori* activity by disk diffusion method with zone diameters of inhibition between 13.5 mm and 40 mm (Table 3). Mean inhibition zone diameter (MIZD) of anti-*H. pylori* activity of testa extract was approximately close to tetracycline and metronidazole and less than amoxicillin (Fig 2). Aqueous extracts of Persian Oak testa layer showed a significant anti-*H. pylori* activity with MIC: 2 μg/mL, MBC: 2 μg/mL by the micro dilution

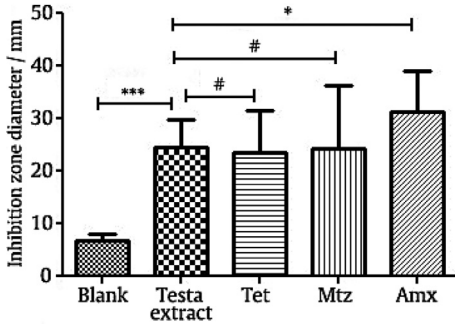


Fig. 2. Comparison of mean inhibition zone diameter of antimicrobial activity against *H. pylori* isolates between extract obtained from testa layer of *Q. brantii* var. *persica* and antibiotics by disk diffusion assay. The results were presented as mean ± standard deviations (SD) and analyzed by a *t*-test and analysis of variance. ***$P < 0.0001$ *vs* control group (blank); *$P < 0.05$ *vs* Amx; #$P > 0.05$ *vs.* Mtz and Tet [Tet: Tetracycline; Mtz: Metronidazole; Amx: Amoxicillin].

method confirming the results of previous tests (agar well diffusion method and disk diffusion method).

4. Discussion

Usage of natural materials has more advantages than other drugs derived from synthetic sources in therapeutic management against the diseases caused by microorganisms such as *H. pylori*.

H. pylori is considered as one of the most common causes of universal infection. This pathogen was ranked as a Class I gastric carcinogen by World Health Organization (WHO) based on the epidemiological studies; It demonstrates its ability to induce carcinogenesis even in the absence of co-carcinogens (Fock, Katelaris & Sugano, 2009).

Prevention and treatment of infections faced with several issues, including antibiotic drug resistance, side-effects of triple or quadruple therapy, compliance with treatment schedule, and lower toxicity of drugs derived from natural sources in comparison to those obtained from synthetic sources; Such issues led to serious attention to novel candidates of herbal sources. Moreover, bacteriological and gastroenterological studies have shown a considerable interest in the pharmacological activities of nat-

Table 3
Mean inhibition zone diameter of antimicrobial extract obtained from testa layer of *Q. brantii* var. *persica* against *H. pylori* isolates compared with different antibiotics by disk diffusion assay.

Samples	Testa extract 2 mg/disc	Tet 30 mg/disc	Mtz 5 mg/disc	Amx 25 mg/disc
Isolate-1	25.5	30	12.5	26
Isolate-2	29	32	19.5	27
Isolate-3	40	24	19	32
Isolate-4	19	21	28	25
Isolate-5	26	19	10	23
Isolate-6	28	21	28	24
Isolate-7	25	24	41.5	16.5
Isolate-8	17	22	20	31
Isolate-9	13.5	29	54	20
Isolate-10	29.5	28	16	50
Isolate-11	24.5	24	25.5	38
Isolate-12	31	10	16	30
Isolate-13	25.5	10	16	30
Isolate-14	27	24	19.5	38
Isolate-15	24.5	35	25	40
Isolate-16	22	33	19.5	35
Isolate-17	19	36	29.5	30
Isolate-18	28	30	35	40
Isolate-19	29	37	46	42
Isolate-20	25	27	52	36
Isolate-21	17	19	20	45
Isolate-22	19.5	10.5	16	27
Isolate-23	19	9	12	30
Isolate-24	21	17.5	11	22
Isolate-25	24.5	14	14	25
Standard	26	24	25	28
MIZD	24.36	23.46	24.22	31.17

Tet: Tetracycline; Mtz: Metronidazole; Amx: Amoxicillin.

ural products against infectious agents (Newman et al., 2007). Many herbal-based therapies work was via synergistic pathways. Several studies support the concept that synergism among compounds of certain plant extracts can overcome resistance noted in monotherapy models or antibiotic resistance (Elfawal, Towler, Reich, Weathers & Rich, 2015; Fairhurst et al., 2012; Miller et al., 2011; Rasoanaivo, Wright, Willcox & Gilbert, 2011; Wright, Linley, Brun, Wittlin & Hsu, 2010; Zhou et al., 2016).

Preliminary study on the prevalence of *H. pylori* gastric infection showed a high prevalence of *H. pylori* gastric infection in the city of Yasuj (68%), the capital of Kohgiluyeh and Boyer-Ahmad in Iran. Overall prevalence of *H. pylori* infection in Iran ranged from 30.6% to 82%, and ranged from 22% to 87.6% in other Eastern Mediterranean Regions (Eshraghian, 2014). Low education (Nouraie et al., 2009), larger family size (Jafar, Jalil, Soheila & Sirous, 2013), and probably increased economic crisis-related stress in Iran (this risk factor is not studied yet) can be related to such high prevalence of *H. pylori* infection.

So far, there are not enough studies done on antibacterial activity of medicinal plants used in traditional medicine system of this province for gastrointestinal disorders. Accordingly, this study is aimed at evaluating the antimicrobial activity of aqueous extracts obtained from testa or seed coat of *Q. brantii* var. *persica* on *H. pylori* isolates from gastric biopsy specimens to identify potentially low-cost sources of therapeutic medicinal plants.

Several natural products have demonstrated anti-*H. pylori* activity. Israel Castillo-Juareza et al. (2009) demonstrated the *in vitro* significant anti-*H. pylori* activity of 53 plants used in Mexican traditional medicine for gastrointestinal diseases. Aqueous extracts of *Artemisia ludoviciana subsp. mexicana, Ludwigia repens, Cuphea aequipetala*, and *Mentha × piperita* (MIC 125 to <250 μg/mL) and also methanolic extracts of *Persea americana, Guaiacum coulteri, Annona cherimola*, and *Moussonia deppeana* (MIC <7.5 to 15.6 μg/mL) showed the highest inhibitory activity.

Phenolic and flavonoid compounds known as secondary metabolite compounds in plants demonstrated a high association with antioxidant activity of most plant extracts. Their potent antioxidant activity is due to presence of an antioxidant and scavenging group (Kessler, Ubeaud & Jung, 2003). Akbartabar, Mirzaei and Mirzaei (2013) demonstrated the potent antioxidant activity of methanolic and aqueous extracts obtained from *Jaft* (testa or seed coat of Persian Oak) using several different *in vitro* antioxidant assays.

Anti-oxidant and anti-lipid peroxidation activity of *Quercus spp.* can be due to the presence of polyphenol and tannin compounds. Extracts obtained from *Q. brantii* have been shown the pharmacological and biological activities such as anti–bacterial effects (Safary, Motamedi, Maleki & Seyyednejad, 2009). In addition, *in vitro* and *in vivo* assessment of acute and chronic toxicity of *Jaft* (Testa) extract obtained from Oak fruit showed its safety for human consumption (Mirzaei et al., 2012).

In the present study, the anti-*H. pylori* activity of aqueous extract obtained from testa layer of *Q. brantii* var. *persica* was assessed by three methods including well diffusion method, broth microdilution assay, and disk diffusion assay *in vitro*. Results of all three tests showed the significant anti-*H. pylori* activity of aqueous extract. Moreover, results of all tests were highly statistically consistent with each other. Zone diameters of inhibition and anti-*H. pylori* activity of aqueous extract increased simultaneously with increasing testa extract concentration.

5. Conclusion

These findings indicated the potential anti-*H. pylori* activity of aqueous extract obtained from testa layer of *Q. brantii* var. *persica*. It is suggested as a natural therapeutic source against the gastric *H. pylori* infection. However, evaluating the *in vivo* activity of this extract is necessary too. Also, other studies are required to detect an anti-*H. pylori* compound existing in testa extract of *Q. brantii* var. *persica* by Mass spectrometry (MS) technique for introducing this assumed compound as a novel natural antibiotic.

Ethical approval

All procedures performed in studies were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards [Ethical Code: Yasuj.MED.1396.A2].

Declaration of Competing Interest

All other authors report no conflicts of interest relevant to this article.

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