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Inhibitory Effect of Partially Purified Compounds from Pomegranate Peel and Licorice Extracts on Growth and Urease Activity of *Helicobacter pylori*

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Abstract

Helicobacter pylori (*H. pylori*) is the main causative agent responsible for chronic gastritis, peptic ulcer, and gastric cancer in humans. An important virulence factor of *H. pylori* is urease that converts urea into ammonia, producing a neutral micro-environment, which allows colonization and survival in the stomach lining. Although various therapies have been introduced for the treatment of *H. pylori* infection, the eradication failure rate stays as high as 5–20% along with frequent recurrence of gastric ulcers even after complete healing. Looking for an alternative mode of treatment using natural products, we investigated the effects of pomegranate peel and licorice extracts that had been fractioned using ethyl acetate and methanol into 4 fractions (F1-F4) on the growth (disc diffusion and minimum inhibitory concentration - MIC) and urease activity of this pathogen. We found that all fractions had antimicrobial activity with the maximum activity observed with the F1 fraction of both pomegranate peel and licorice extracts with growth inhibitory zones of 33 mm and 26 mm, and MICs of 2 and 1 mg/ml, respectively. The F2 and F4 fractions of licorice extract, and F3 of pomegranate peel extract showed MIC values of 4 mg/mL, whereas the F4 fraction of pomegranate peel extract and F3 of licorice extract showed MIC values of 8 mg/ml. The F1 and F2 fractions of pomegranate peel caused 80 and 91% inhibition of urease activity and the F1 fraction of licorice caused total inhibition (100%) of *H. pylori* urease activity at MIC values. In conclusion, these results indicate that both pomegranate and licorice contain important bioactive compounds against *H. pylori* and should be examined further to help in the fight against this important pathogen.

Keywords: *Helicobacter pylori,* Pomegranate peel, Licorice, Urease, Gastritis

1. Introduction

Helicobacter pylori (*H. pylori*) is an important pathogen that selectively colonizes the epithelium of the stomach and duodenum (Ruggiero, 2010), which leads to gastritis, peptic ulcer, and gastric cancer in humans (Sokolova & Naumann, 2022; Abu-Ahmad *et al*., 2011). The key virulence factor produced by *H. pylori* is urease (Valenzuela-Valderrama *et al*., 2019), which converts urea into ammonia and enables the microorganism to colonize the stomach lining (Bansil *et al*., 2013). Other virulence factors include cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin (vacA) proteins (Wen & Moss, 2009) which are responsible for the pathogenicity of *H. pylori* (Radosz-Komoniewska *et al*., 2005). CagA-positive *H. pylori* strains have been associated with interleukin-8 (IL-8) induction in gastric epithelium. Increased production of IL-8 causes neutrophilic infiltration into gastric epithelium leading to epithelial inflammation and disruption of the tight junctions and cell integrity of the stomach (Huang *et al*., 2014). The *vacA* gene encodes VacA protein which can remain on the bacterial surface (Foegeding *et al*., 2016) and cause cell death (Chauhan *et al*., 2019).

Triple therapy with a proton pump inhibitor (PPI) and the antimicrobials amoxicillin and clarithromycin is a recent therapeutic regimen for *H. pylori* eradication (Puig *et al*., 2016). It was found to be highly effective in the long-term treatment of disease in 80% of the patients (Juergens *et al*., 2010). Although this mode of therapy shows some related side effects such as diarrhea, colitis (Cremonini *et al*., 2002), and low eradication of *H. pylori* (Perri *et al*., 2001), it led to the absence of the pathogen (Moss and Calam, 1992). However, it failed to exterminate the bacteria in 10-20% of patients (Choi *et al*., 2012) due to strains resistant to clarithromycin.

Quadruple therapy was then introduced as a better regimen for treating *H. pylori* infection (Kanizaj and Kunac, 2014) with amoxicillin, metronidazole, omeprazole, and bismuth subnitrate (Lu *et al*., 2013). Quadruple therapy is usually associated with high levels of antibiotic resistance (Ndip *et al*., 2008; Tanih *et al*., 2010), although it is reported to be 90% effective (Tanih *et al*., 2010). However, the eradication failure rate stays as high as 5–20% along with frequent recurrence of gastric ulcers even after complete healing. The problem of incomplete/partial treatment that occurs with triple and quadruple therapy, in addition to the possible side effects (Li *et al*., 2005), has led to the search for new chemical

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compounds with bacteriostatic or bactericidal effects against *H. pylori* (González *et al*., 2019) in order to overcome these challenges. There is growing interest in looking at alternative treatments using natural products that are relatively nontoxic and cheap (Ayala *et al*., 2014).

Several natural materials are used in folk medicine for the treatment of bacterial infections and examples of different natural compounds that exhibit activity against *H. pylori* include broccoli, curcumin, *Nigella sativa* or black caraway, olive oil and mume (Murali*et al*., 2014). Many of these natural products have been shown to have phenolic phytochemicals such as cinnamic acids, cinnamaldehydes, coumarins, phenolic acids, capsaicin, flavonoids, and tannins (Tabak *et al*., 1996; Jones *et al*., 1997; Bae *et al*., 1999; Graham *et al*., 1999; Tabak *et al*., 1999; Yanagawa *et al*., 2003).

Pomegranate (*Punica granatum* L.) belongs to the genera *Punica* and the family *Punicaceae* (Chen *et al*. 2019), and the fruits, leaves, flowers, and bark of this plant have been used in various fields of traditional medicine (Mekni *et al*., 2013) of the Middle East, India, China, Pakistan, and many other countries (Zhicen, 1987; Kapoor, 1990). It has been used as an antiparasitic agent, and to treat various illnesses including diarrhea, diabetes (Moghaddam *et al*., 2013), rheumatism, sore throats and to decrease inflammation (Arun and Singh, 2012). In addition, pomegranate has been used to treat infections caused by *Salmonella, Shigella, Proteus, Klebsiella, Escherichiacoli, Pseudomonas, Vibrio cholerae,* and *Staphylococcus aureus.* Moreover, pomegranate exocarp anthocyanin methanol extract combined with metronidazole has been shown to significantly decrease the colonization of *H. pylori* in rats (Ragab, A.E. *et al*, 2022). Pomegranate peels represent approximately 40% of the whole fruit and contain several phytochemicals including punicalin, punicalagin, granatin B, gallagyldilactone, punicalagin, pedunculagin, and tellimagrandin (Cerda *et al*., 2003; Seeram *et al*., 2005; Jurenka, 2008; Magangana *et al*., 2020). The antioxidant activity of pomegranate peels has been linked with its phenolic components such as gallotannins, anthocyanins, ellagitannins, gallagyl esters, hydroxycinnamic acids, dihydroflavonol and hydroxybenzoic acids (Fischer *et al*., 2011).

Licorice (*Glycyrrhizauralensis)* is another important medicinal plant, and the roots, stolons and rhizomes of this plant have also been used in traditional medicine. The major phenolic compounds of licorice include glycosides of liquiritigenin (4,7-dihydroxyflavanone) and isoliquiritigenin (2,4,4-trihydroxychalcone) (Bethapudi *et al*., 2022). Licorice extract has been investigated as a substitute to bismuth that is normally used in quadruple therapy (Li *et al*., 2018). Bismuth protects against acid and pepsin secretions by covering the location of the lesion and promoting mucous secretion (Sreeja *et al*., 2020).

Given the activities and benefits of pomegranate peel and licorice, in the current study we investigated the effect of pomegranate peel and licorice on the growth and urease activity of *H. pylori*.

2. Materials and Methods

2.1. Organism and growth conditions

Helicobacter pylori was obtained from American Type Culture Collection (ATCC 700392). Columbia blood agar base enriched with 7% horse serum and Brucella broth were used to grow and maintain *H. pylori* cultures. Incubation was done under microaerophilic conditions using a campygen pack at 37 ºC for 48-72 hours

2.2. Pomegranate peel and licorice extraction procedure

Pomegranate peel was dried at room temperature for 7 days and then grinded to a powder. Licorice powder was purchased from local markets in Jordan. The plant extraction method was a modification of the protocol used by Ajaikumar *et al.* (2005). Twenty grams of both powdered pomegranate peel and licorice were extracted with 200 ml of methanol and then mixed using a magnetic hot plate at 45 ºC for 3 hr. A rotary evaporator was used at 45 ºC to concentrate the extract to 100 mg and dissolve it in 1 ml dimethylsulfoxide (DMSO). Fractionation of concentrated extract was performed depending on the polarity of their compounds using methanol and ethyl acetate solvents by Isolera Prime™ Flash Purification System at flow rates 18 ml /min, followed by drying it using nitrogen gas then stored in the refrigerator until use. Each fraction (0.1 g) was dissolved in1 ml of DMSO in an Eppendorf tube. Doubling dilutions were performed for each crude extract using DMSO as the following 1:1, 1:2, 1:4 and 1:8. Thin layer chromatography (TLC) was used to determine the number of fractions (F) that found in pomegranate peel and licorice extracts using methanol solvent. The partially purified fractions were dissolved in DMSO (0.1 g of each fraction was dissolved in 1 ml of DMSO).

2.3. Bacterial growth inhibition

The Disk diffusion method was performed to investigate the bacterial growth inhibition to determine the effect of pomegranate peel and licorice crude extracts and their fractions on *H. pylori* activities *In vitro* according to Zhang *et al*. (2013).

H. pylori suspension (0.1 ml in liquid Brucella medium) (1×10^8 CFU/ml) was inoculated on the Columbia agar surface. Sterile paper disks (6 mm) were impregnated with10 µl of different concentrations from each crude extract of pomegranate peel and licorice. The negative control used was the DMSO and the positive control was ampicillin (100 mg/ml). All test plates were incubated in a microaerophilic atmosphere. The inhibitory zone around each disk was measured

2.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the partially purified fractions against Helicobacter pylori

The MIC and MBC were determined using a broth microdilution method (Weseler *et al*., 2005). The partially purified fractions of pomegranate peel and licorice were diluted in sterile water to obtain a series of concentrations for each fraction 32.0, 16.0, 8.0, 4.0, 2.0, and 1.0 mg/ml. Separately, 100 µl of each concentration was added to 96 well plates containing 100 µl of *H. pylori* (1×10^8 CFU/ml) supplemented with 7% horse serum. Plates were incubated

under the microaerophilic condition at 37 ºC for a period of 20±2 h. A tetrazolium salt p-iodonitrophenyltetrazolium violet (INT) was used as an indicator of bacterial growth in which INT reduced to violet color formazan. The MICs were determined as the lowest concentration of each fraction showed no detectable *H. pylori* growth. After an incubation period, 10 µl from each fraction was spread onto the surface of the Columbia agar plate to determine the MBC values. The MBCs were determined as the lowest concentration of each fraction that showed no detectable *H. pylori* growth.

2.5. Growth curve of Helicobacter pylori

The partially purified fractions of pomegranate peel and licorice at their MIC concentrations were evaluated for their impact on *H. pylori* growth. Briefly, separately 100 µl of each concentration was added into a 96-well plate containing 100 µl of *H. pylori* (1×10^8 CFU/ml) supplemented with 7% horse serum. Brucella broth was used as a negative control. Plates were incubated under the microaerophilic condition at 37 ºC, then optical density (OD) at 600 nm was measured at different intervals.

2.6. Effect of partially purified compounds on urease activity of Helicobacter pylori

Forty ml broth cultures (1×10^8 CFU/ml) were harvested by centrifugation at 5000 rpm for 10 min at 4 °C. Cells were suspended in 4 ml phosphate buffer pH 7.4 containing 5 mg protease inhibitor. This suspension was disrupted by four 15-sec periods of ultrasonication punctuated with 15-sec rest periods in an ice bath. Following centrifugation at 5000 rpm for 10 minutes at 4 ºC, the supernatant was filtered using 0.2 µm membrane filters. Protein concentrations in filtrated solution were determined using the Bradford protein assay (Bardford, 1976).

Ammonia production was used to assess the inhibition of partially purified compounds on urease activity of *H. pylori*(Matsubara *et al*., 2003 and Shin *et al*., 2003): using 96 well plates, 30 µl of each purified fraction was mixed with 30 µl of urease reaction solution (final concentration 200 µg/ml) and incubated at 37 °C for 10 min, distilled water was also used as control. Fifty µl of urea reagent (2% urea and 0.03% phenol red in phosphate-buffered saline, pH 6.8) was added to initiate the urease reaction. Ammonia concentration was determined by measuring the absorbance at 520 nm.

Inhibition rate was calculated using the formula: (Inhibition rate) % = ((OD520 control - OD520 sample)/OD520 control) * 100.

2.7. Statistical analysis

All assays were performed in triplicate and the results were expressed as the mean value. Results of the growth inhibition assay, MIC and MBC, and urease assay were analyzed using a one-sample students T-test. Growth curve effects for partially purified fractions were analyzed using one-way analysis of variance (ANOVA) by performing the Least Significant Difference (LSD) multiple comparison tests on the data means. The Statistical Package for the Social Sciences (SPSS) version 20.0 software was used for the statistical analyses. *P*-value ≤ 0.05–0.01 was considered significant and *P*-value ≤ 0.01 was considered highly significant in all data analyses.

3. Results

Crude extracts of pomegranate peel and licorice at different concentrations were found to inhibit the growth of *H. pylori* using the disk diffusion method (*P*-value= 0.005 and *P*-value= 0.001 for pomegranate peel and licorice, respectively). The maximum inhibition zone was found at 100 mg/ml for both pomegranate peel and licorice crude extracts (Table 1). Pomegranate peel extract showed an inhibition zone of about 29 mm, whereas the inhibition zone for licorice was 21 mm. DMSO and ampicillin were used as negative and positive controls, respectively (Table 1).

Table 1: Growth inhibition zone of crude extracts of pomegranate peel and licorice against *H. pylori*.

Concentrations	Inhibition zone of pomegranate peel (mm)*	Inhibition zone of Licorice (mm) **
100 mg/ml	29.3	21.3
1:1	28.7	19
1:2	20.7	18.3
1:4	16.7	16
1:8	10	13.6
Ampicillin	78.3	78.3
DMSO	0	0

**P*-value= 0.005 ***P*-value= 0.000

Pomegranate and licorice extracts were fractioned using ethyl acetate and methanol solvents by Isolera Prime™ Flash Purification System. Four fractions (F1-F4) for each licorice and pomegranate peel were obtained according to their polarity. These fractions were evaluated for their inhibitory influence on *H. pylori* growth by using the disk diffusion method. The diameters of inhibitory zones of the partially purified fractions are shown in Table 2, and it can be seen that the maximum antimicrobial activity was found with the F1 fraction for both pomegranate peel and licorice. The F1 fraction of pomegranate peel extract showed an inhibition zone of 34 mm, whereas that of licorice showed an inhibition zone of 27 mm. The negative control, DMSO, did not affect the growth of *H. pylori*.

Table 2: Growth inhibition zone of pomegranate peel and licorice fractions and DMSO against *H. pylori*.

Pomegranate peel and Licorice fractions	Inhibition zone of pomegranate peel (mm)*	Inhibition zone of Licorice (mm)*
F1	33.7	26.7
F2	24	13.7
F3	20.7	12
F4	15.7	16
Ampicillin	78.3	78.3
DMSO	0	0

**P*-value= 0.008 **P*-value= 0.014

Table 3 shows the results of the MIC and MBC for the partially purified compounds that were determined using the microdilution method. The F1fraction of licorice extract showed the lowest MIC value of 1 mg/mL, whereas the F1 fraction of pomegranate peel extract showed an

MIC value of 2 mg/ml. However, the F2 and F4 fractions of licorice extract, and F3 fraction of pomegranate peel extract showed MIC values of 4 mg/ml, whereas F4 of pomegranate peel extract and F3 of licorice extract showed MIC values of 8 mg/ml. The DMSO that was used to dissolve the isolated active compounds showed no interference with the MIC results. There were no significant differences between the fractions of both pomegranate peel and licorice (*P*-value= 0.094 for pomegranate peel fractions at their MIC and MBC values and *P*-value= 0.06 and 0.074 for licorice fractions at their MIC and MBC values respectively).

Table 3. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the partially purified fractions on *Helicobacter pylori* growth(mg/ml)

	Pomegranate peel		Licorice	
Fraction	MIC	MBC	MIC	MBC
F1	2	4	1	1
F2	16	32	4	8
F3	4	8	8	16
F4	8	16	4	8
P-value= 0.094		*P*-value= 0.06(MIC) and 0.074(MBC)		

The F1 fractions of both pomegranate peel and licorice at their MIC values inhibited the growth of *H. pylori* (Figures 1 and 2). Complete (100%) growth inhibition of *H. pylori* using both pomegranate peel and licorice was obtained after 24-hour incubation under microaerophilic conditions.

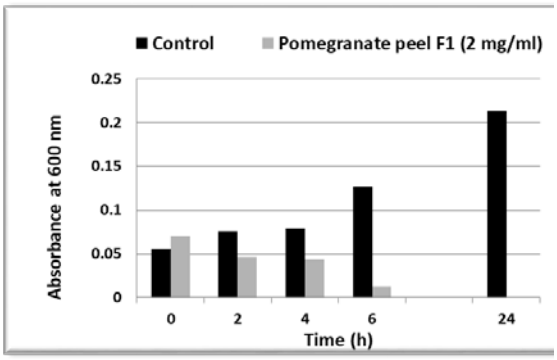

Figure 1. The effect of F1 from pomegranate peel on the growth of *Helicobacter pylori* (*P-value*= 0.00)

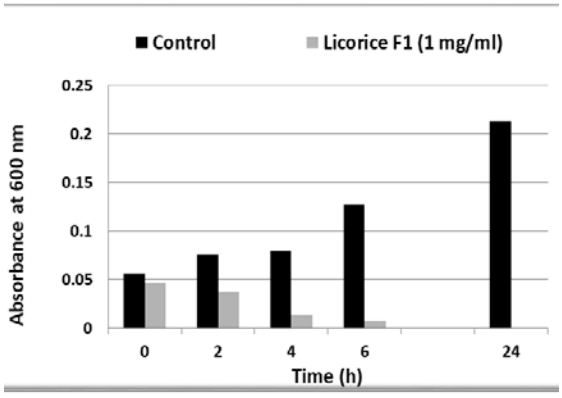

Figure 2. The effect of F1 from licorice on the growth of *Helicobacter pylori*(*P-value*= 0.019)

The effects on inhibition of*H. pylori* urease activity using different fractions of pomegranate peel and licorice at their MIC values are shown in Table 4. The F2 fraction of pomegranate peel caused 91% inhibition while F1 of licorice caused 100% enzyme inhibition (Table 4). However, the F1 fraction of pomegranate peel inhibited the enzyme by 80%.

Table 4. Percentage inhibition of *H. pylori* urease enzyme using pomegranate peel and licorice fractions.

Fractions	Urease inhibition (%) Pomegranate peel	Urease inhibition (%) Licorice
F1	79.6	100
F2	91	20.1
F3	8.8	43.9
F4	4.8	55.7

*Urease activity of *H. pylori* =

4. Discussion

With increasing problems associated with antimicrobial resistance together with the added challenges of currently available eradication therapies of *H. pylori* infections, such as high cost and poor compliance of patients, it is of utmost importance to develop novel antibacterial treatments using natural materials against this very important pathogen. A variety of natural compounds have been reported to exhibit antimicrobial activity against *H. pylori* including olive oil, *Nigella sativa*, broccoli, mume,and curcumin (Murali *et al*., 2014), pomegranate (Hajimahmoodi *et al* 2011; Moghaddam, 2011) and licorice (Fukai *et al* 2001). Indeed, both pomegranate and licorice have been used in traditional medicine throughout history to provide relief from various gastrointestinal problems ranging from diarrhea, epigastric pain to peptic ulcers. This encouraged us to study the effects of these phytotherapeutic agents on the growth and urease production of *H. pylori*. Our results indicate that indeed, crude extracts of both pomegranate peel and licorice were active against *H. pylori*. The crude extract of pomegranate peel showed a greater degree of inhibition (29mm) of growth of *H. pylori* than that of licorice (21mm). Our results agree with those of Hajimahmoodi *et al* (2011) who reported that crude extracts of pomegranate peel showed inhibition zone diameters ranging from 16 to 40 mm. Another study showed that the highest inhibition zone of pomegranate peel was 27.96 mm at 2 mg of crude extract/disc (Moghaddam, 2011). In addition, Fukai *et al* (2001) reported that licorice fractions showed inhibition diameters ranging from 11.5 to 19 mm, which is slightly less than the growth inhibitory zones reported in the results of the current study.

In addition, we found that all of the four different isolated methanolic fractions inhibited the growth of *H. pylori* and *i*nterestingly the F1 fractions from both pomegranate peel and licorice showed greater activity producing larger growth inhibitory zones (33.7 and 26.7 mm, respectively) than the crude extracts. We also found that the F1 fraction of pomegranate peel and licorice exhibited the highest bacteriostatic and bactericidal activity (MIC and MBC of 2mg/ml and 4mg/ml, respectively for pomegranate; 1mg/ml for licorice) in comparison to the other fractions tested, using the modified broth dilution method. Moghaddam (2011), on the other hand, reported that the MIC and MBC for pomegranate peel was much lower (312.5 µg/ml and 338.5

µg/ml, respectively) using the agar dilution method. In contrast to our results, Jafarian and Ghazvini, (2007) reported that MIC for licorice against *H. pylori* was much higher than the results of the current study (range of 50-400 mg/ml, compared to 1mg/ml for the F1 fraction in the current study).

H. pylori is known for its high activity of urease enzyme which protects the bacterium from gastric secretions. Both pomegranate peel and licorice were found to inhibit urease activity. The F1 and F2 fractions of pomegranate peel showed the greatest inhibitory effects with 80 and 91% inhibition of enzyme activity, respectively at MIC values of 2mg/ml. Nabati *et al*. (2012) reported that a crude extract of pomegranate peel causes almost total inhibition (99.9%) of urease activity of this pathogen. This effect may be due to the presence of ellagic acid derivatives such as the ellagitannins, punicalagin, and punicalin (Cerda *et al*., 2003; Larrosa *et al*., 2006) and catechins (Julie Jurenka, 2008). Furthermore, we found that the F1 fraction of licorice at its MIC value had the strongest activity with 100% inhibition of urease activity. This effect may be due to the presence of flavonoid compounds (Eloff *et al*., 1998; Cowan *et al*., 1999). Complete or partial inhibition of urease activity would result in decreased ability of *H. pylori* to survive the acidity of the gastric secretions.

As a vital factor of virulence, the effects of pomegranate peel and licorice on inhibition of urease activity in the current study are of particular significance. Without this vital enzyme, the ability of *H. pylori* to establish infection would be totally compromised. Indeed, in a gnotobiotic piglet model, *H. pylori* mutants that were unable to produce urease could not colonize the gastric mucosa and were unable to cause infection (Eaton *et al* 1991). It comes as no surprise that the production of urease has been suggested an important target for innovative alternate antimicrobic regimens to thwart the unending problem of drug resistance of this important pathogen (Tarsia *et al* 2018). Urease activity is not only important in allowing *H. pylori* to colonize and infect the gastric mucosa but the resulting increased pH also decreases the viscoelastic properties of the gastric mucus which would permit greater mobility of *H. pylori* and allow easier penetration and attachment to the host epithelial cells (Celli *et al* 2009).

The results of the current study clearly indicate that exposure of *H. pylori* to extracts of pomegranate and licorice resulted in inhibition of growth and urease activity of this pathogen. One limitation of the current study is that the isolated fractions of both plants were not characterized further. This needs to be done in order to identify the component(s) responsible for the observed inhibitory effects. Furthermore, future studies should focus on *in vivo* testing of these extracts in animal models of *H. pylori* infection to correlate these *in vitro* findings.

In addition to the bactericidal effects and urease inhibiting properties, pomegranate peel and licorice possess other properties that promote gastric health. For example, pomegranate possesses antineoplastic effects since it inhibits proliferation of cancer cells causing cell cycle disruption and apoptosis of cancer cells, and also inhibits angiogenesis (Lansky *et al*., 2007; Amin *et al*., 2009; Adhami *et al*., 2009). Indeed, it has been reported to be effective against different types of cancer including colon cancer (Amin *et al*., 2009), and this has been attributed to the antioxidant and anti-proliferative effects (Amin *et al*., 2009; Adhami *et al*., 2009). The antioxidant properties of pomegranate peel (Iqbal *et al*., 2008) would prevent lipid peroxidation which results in the formation of reactive oxygen species and free radicals that are associated with carcinogenesis (Shahidi, 1997; Siddhuraju and Becker, 2003). Furthermore, the flavonoid-rich fractions of licorice not only exhibit anti-*H. pylori* activity (Eloff *et al*., 1998; Cowan *et al*., 1999), but also anti-ulcer activity (Aly *et al*., 2005; Shibata and Saitoh, 1973) due to their antioxidant, anti-inflammatory, and anticarcinogenic effects (Park *et al* 2014). Not surprisingly, licorice has been used for many decades to treat gastric ulcers based on three lines of evidence that confirm that licorice-derived compounds have ulcer healing ability (Duke 1985). Firstly, licorice-derived products induce increases in prostaglandin levels in the digestive system and have been reported to enhance increased secretion of protective gastric mucin. Secondly, licorice is thought to play a role in increasing the life span of superficial gastric cells and thus slowing down the process of gastric lesion development. Thirdly, it has pepsin inhibitory properties which would lead to less damage to the gastric epithelium. Indeed, the licorice-derived carbenoxolone has been used to promote healing of gastric ulcers since its development in the 1960s (Dehpour 1994). In addition, the many flavonoids with one or more isoprenoid groups that have been isolated from *Glycyrrhiza* species contribute to the anti-*H. pylori* activity of licorice (Nomura and Fukai,1998; Yamada *et al*., 1998; Krausse *et al*., 2004).

5. Conclusion

Taken together, the results of the current study and the findings reported in the available literature indicate that both pomegranate peel and licorice not only kill *H. pylori*, but have protective effects on the gastric mucosa. Our results are of significance in highlighting the promising potential of pomegranate and licorice as safe sources to control *H. pylori* infections. Furthermore, pomegranate peel and licorice fractions can be further fractionated to determine the most active chemical compound(s) and test them against *H. pylori* both *In vivo* and *In vitro* and compare the effectiveness with the antibiotics in use.

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