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ORIGINAL ARTICLE

Anti-*Helicobactor pylori* activity of some Jordanian medicinal plants

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Abstract

Context: Natural flora are considered a major source of new agents for the treatment of *Helicobactor pylori*. The plants used in this study were selected based on previous traditional use.

Objective: In this study, we evaluated the effect of extracts of 16 medicinal plants grown in Jordan against clinical isolates of *H. pylori*.

Materials and methods: Tested plant extracts included *Aloysia triphylla* (L'Her.) Britton (Verbenaceae), *Anethum graveolens* L. (Apiaceae), *Artemisia inculata* Delile (Asteraceae), *Capparis spinosa* L. (Capparaceae), *Crataegus aronia* (L.) Bosc ex. DC. (Rosaceae), *Inula viscose* (L.) Ait (Asteraceae), *Lavandula officinalis* Chaix. (Lamiaceae), *Lepidium sativum* L. (Cruciferae), *Origanum syriaca* L. (Lamiaceae), *Paronychia argentea* Lam. (Caryophyllaceae), *Passiflora incarnate* L. (Passifloraceae), *Psidium guajava* L. (Myrtaceae), *Sarcopoterium spinosum* (L.) Spach (Rosaceae), *Sesamum indicum* L. (Pedaliaceae), *Urtica urens* L. (Urticaceae) and *Varthemia iphionoids* Boiss (Asteraceae). Clinical isolates of *H. pylori* were tested *in vitro* for susceptibility to each of the above plant crude extracts using disk diffusion method, and the MIC value was determined for each plant extract using the serial dilution method.

Results: Results showed that ethanol extracts of most medicinal plants exerted cytotoxiciy against *H. pylori* isolates. Among the tested plant extracts, *A. triphylla* (MIC: 90 μg/mL, MBC: 125 μg/mL) and *I. viscosa* (MIC: 83 μg/mL, MBC: 104 μg/mL) showed the strongest activity against both isolates of *H. pylori*.

Discussion and conclusion: Jordanian medicinal plants might be valuable sources of starting materials for the synthesis of new antibacterial agents against *H. pylori*.

Keywords

Aloysia triphylla, *H. pylori*, *Inula viscose*, Jordan, MIC

History

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Introduction

Helicobacter pylori infection is one of the most prevalent human infections and has been implicated as a predisposing factor in gastric cancer, chronic active gastritis, duodenal ulcer, gastric ulcer and gastric lymphoma (De Francesco et al., 2010; Kawai et al., 2010; Krueger et al., 2011; Touati, 2010). The infection is currently endemic worldwide with higher prevalence in developing regions in South America, Africa and Asia compared with the developed industrial world (De Francesco et al., 2010). Eradication of *H. pylori* can be achieved through either triple therapy or quadruple therapies containing combinations of antibiotics along with strong acid suppressive agents. However, the increased rate of antibiotics resistance, along with possible adverse effects, cost of combination therapy, increased reoccurrence of *H. pylori* infection after eradication and limited compliance of patients provide a strong reason for the search for new drugs with similar or more beneficial antibiotic properties but with reduced side effects (Alzoubi et al., 2007; Di Mario et al., 2006; Masadeh et al., 2013; O'Gara et al., 2000).

The rational testing of bioactive products from traditional medicine is an established strategy for the discovery of novel compounds with potent and therapeutically useful antimicrobial activities. In the present study, we evaluated the antimicrobial activity of 16 Jordanian medicinal plant extracts against two clinical isolates of *H. pylori*. The selection of plants for this study was based on their known medicinal use for the management of various infectious diseases, particularly of bacterial origin. These include skin infections, dysentery, diarrhea, eye infection and venereal diseases (Al-jarwani & Khalifeh OStamatis, 1936; Jabour, 1983; Karim & Quraan, 1986; Kotb, 1985).

Materials and methods

Plant materials used in the current study were collected during the months of May through August of the summer season of 2010 from various locations of the North of Jordan. Taxonomic identification of the plants was confirmed by Professor Jammel Lahaam, Department of Biological

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Sciences, Faculty of Science, Yarmouk University, Irbid-Jordan. A voucher specimen from each plant was deposited at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid-Jordan. The plant materials were shade-dried and ground in a Wiley grinder (Model 5657 HAAN, Germany) with a 2 mm diameter mesh. Powdered plant materials (50 g/each) were percolated in EtOH (95%). The combined ethanol extracts were concentrated in vacuum to give a dried extract, which was subjected to further fractionation.

Microbial culture and growth conditions

H. pylori was cultured from patients presenting with gastro-duodenal pathologies attending King Abdullah University Hospital (KAUH) in Irbid-Jordan after informed consent was obtained from the patients. Briefly, biopsies were inoculated on Columbia agar base plates (Conda Pronadisa, Spain) supplemented with 7% sterile defibrilated sheep blood, amphotericin B (250 mg) and *Campylobacter* supplements; trimethoprim (5 mg/L), vancomycin (10 mg/L) and polymyxin B (2500 units/L). Plates were incubated at 37 °C for 3–5 days in a microaerophilic environment (10% carbon dioxide, 5% oxygen) (Anaerocult, Darmstadt, Germany). Two isolates were obtained and characterized and were subjected to antimicrobial assays.

Antimicrobial susceptibility test

Sterile, 5-mm diameter filter paper discs were impregnated with 60–100 µg of the tested plant extract in DMSO and placed in duplicates onto Mueller-Hinton agar (MHB). The surface was then spread with 0.2 mL of microorganism culture (ca. 10^8 cells/mL) and the plates were incubated for 24 h at 37 °C. The experiments were carried out in duplicate. The results (mean of three independent experiments) were recorded by measuring the zones of growth inhibition surrounding the discs. Negative control discs contained only DMSO. The following antibiotics were tested as positive controls: amoxicillin (10 µg/disc), tetracycline (30 µg/disc), metronidazole (5 µg/disc) and clarithromycin (20 µg/disc). After incubation, extract concentrations that showed a diameter of inhibition of at least 15 mm were considered to be active.

Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration was determined by serial dilution method according to the procedures reported previously [Clinical and Laboratory Standards Institute (CLSI), 2012]. Briefly, stock solutions of all extracts were passed through a pyrogenic filter to sterilize the solution and serially diluted to a range of concentrations (1000–1.58 mg/mL). The 96-well plates were prepared by dispensing into each well 100 µL of an appropriate medium, test extract and 20 µL of the inoculum. A standard nutrient broth (MHB) was employed for the bacterial assays. The growth of the microorganism was determined by turbidity. Clear wells indicated the absence of bacterial growth. For every experiment, a sterility check (50% DMSO and medium), negative control (50% DMSO, medium and inoculum) and positive control (50% DMSO, medium, inoculum and Clarithromycin) were included. The microtitre plates were incubated at 37 °C for 24 h and were examined for growth in daylight. The MIC of the preparations was the lowest concentration in the medium that completely inhibited visible growth. The solvent value was deducted accordingly to obtain the results of activity. All experiments were performed in triplicate.

To determine MBC, tubes that showed no visible bacterial growth during MIC testing were sampled (100 µL) and impregnated on to MBH in sterile wells. Plates were incubated at 37 °C for 24 h in microaerophilic environment (10% carbondioxide, 5% oxygen) (Anaerocult, Darmstadt, Germany). The MBC was considered as the lowest concentration that yielded no bacterial growth in this experimental setting.

Results

In this study, we investigated the *in vitro* antibacterial activity of 16 ethanol extracts of Jordanian medicinal plants (Table 1) against two clinical isolates of *H. pylori*. Results shown in Table 2 indicate that plant extracts induced antimicrobial activity against both isolates of *H. pylori*. Both isolates responded equally to the conventionally used antibiotic such as amoxicillin, tetracycline, metronidazole or clarithromycin. DMSO was used as negative control and showed no activity. The clinical isolates of *H. pylori* were most sensitive to ethanol extracts of *Aloysia triphylla* (L'Her.) Britton (Verbenaceae) and *Inula viscose* (L.) Ait (Asteraceae). They were also moderately sensitive to extracts of *Paronychia argentea* Lam. (Caryophyllaceae), *Passiflora incarnate* L. (Passifloraceae) *Artemisia inculata* Delile (Asteraceae), *Varthemia iphionoids* Boiss (Asteraceae), *Sarcopoterium spinosum* (L.) Spach (Rosaceae), *Psidium guajava* L. (Myrtaceae), *Lavandula officinalis* Chaix. (Lamiaceae), *Origanum syriaca* L. (Lamiaceae), *Lepidium sativum* L. (Cruciferae), *Anethum graveolens* L. (Apiaceae), *Sesamum indicum* L. (Pedaliaceae) and *Urtica urens* L. (Urticaceae). Additionally, clinical isolate 2 was moderately sensitive *Capparis spinosa* L. (Capparaceae). However, ethanol extracts of *Crataegus aronia* (L.) Bosc ex. DC. (Rosaceae) and *C. spinosa* were weakly active against both isolates.

The MIC for both *A. triphylla* and *I. viscose* were determined. Results show that the average MIC of *A. triphylla* was 90 µg/mL, and for *I. viscose* was 83 µg/mL for *H. pylori* isolates. On the other hand, their MBC values were 125 and 104 µg/mL, respectively.

Discussion

The findings of this study show the activity of extracts of some Jordanian medicinal plants to two clinical isolates of *H. pylori*. The two most active plants extracts were those of *A. triphylla* and *I. viscose*.

The antibacterial effects of *A. triphylla* might be attributed to the high leaf contents of polyphenolic compounds including verbascosides and luteolin-7-diglucuronide (Carnat et al., 1999). Verbascosides were previously shown for their antibacterial activity against *Proteus Mirabilis* and

Table 1. List of medicinal plants used in the screening for antibacterial activities.

Scientific name	Family	Part used	Voucher specimen number	Yield (g/kg)
Aloysia triphylla (L'Her.) Britton	Verbenaceae	Leaf	73	59.0
Anethum graveolens L.	Apiaceae	Seed	62	47.0
Artemisia inculata Delile	Asteraceae	Aerial parts	104	131.0
Capparis spinosa L.	Capparaceae	Leaf	115	120.0
Crataegus aronia (L.) Bosc ex. DC.	Rosaceae	Leaf and fruit	89	47.4
Inula viscosa (L.) Ait	Asteraceae	Leaf	112	216.2
Lavandula officinalis Chaix.	Lamiaceae	Leaf	85	30.4
Lepidium sativum L.	Cruciferae	Seed	88	31.8
Origanum syriaca L.	Lamiaceae	Leaf	52	47.2
Paronychia argentea Lam.	Caryophyllaceae	Aerial parts	78	20.8
Passiflora incarnata L.	Passifloraceae	Flower	156	10.9
Psidium guajava L.	Myrtaceae	Leaf	67	166.0
Sarcopoterium spinosum (L.) Spach.	Rosaceae	Root	51	263.4
Sesamwn indicum L.	Pedaliaceae	Leaf	94	50.8
Urtica urens L.	Urticaceae	Leaf	76	20.2
Varthemia iphionoides Boiss	Asteraceae	Aerial parts	97	93.0

Table 2. Antibacterial activity of sixteen ethanol plants extracts with different concentrations against *H. pylori* clinical isolates.

	Diameter of zone of inhibition (mm)													
	H. pylori Isolate 1							*H. pylori* Isolate 2						
Plant/concentration in μg/mL	Stock	C1	C2	C3	C4	C5	C6	Stock	C1	C2	C3	C4	C5	C6
Aloysia triphylla	46	34	28	22	12	0	0	36	28	20	15	0	0	0
Anethum graveolens	23	0	0	0	0	0	0	35	25	18	15	0	0	0
Artemisia inculata	30	25	20	15	0	0	0	31	27	20	17	0	0	0
Capparis spinosa	0	0	0	0	0	0	0	20	16	11	0	0	0	0
Crataegus aronia	0	0	0	0	0	0	0	25	15	0	0	0	0	0
Inula viscosa	40	35	23	18	0	0	0	40	33	28	18	0	0	0
Lavandula officinalis	32	28	23	18	0	0	0	35	25	17	0	0	0	0
Lepidium sativum	33	29	23	18	0	0	0	37	28	22	15	0	0	0
Origanum syriaca	30	24	15	0	0	0	0	28	21	18	15	0	0	0
Paronychia argentea	20	15	0	0	0	0	0	18	0	0	0	0	0	0
Passiflora incarnata	29	23	20	0	0	0	0	18	11	0	0	0	0	0
Psidium guajava	33	25	18	12	0	0	0	28	18	0	0	0	0	0
Sarcopoterium spinosum	32	24	15	9	0	0	0	30	25	17	0	0	0	0
Sesamum indicum	30	26	22	13	0	0	0	28	25	18	15	0	0	0
Urtica urens	20	15	0	0	0	0	0	18	15	11	0	0	0	0
Varthemia iphionoides	25	20	13	0	0	0	0	30	26	20	16	0	0	0
Amoxicillin				55							62			
Clarithromycin				75							0			
Metronidazole				17							0			
Tetracycline				20							32			

Stock = 100 000 μg/mL, C1 = 50 000 μg/mL, C2 = 25 000 μg/mL, C3 = 12 500 μg/mL, C4 = 6250 μg/mL, C5 = 3125 μg/mL, C6 = 1582.5 μg/mL, amoxicillin = 10 μg/disc, metronidazole = 5 μg/disc, clarithromycin = 20 μg/disc, and tetracycline = 30 μg/disc.

Staphylococcus aureus (Avila et al., 1999; Didry et al., 1999). Their mode of action is thought to be associated with inhibition of bacterial protein synthesis in a manner similar to that of the conventional antibiotic chloramphenicol (Avila et al., 1999). Additionally, luteolin-7-diglucuronide is a major flavonoid that was characterized for its potent antibacterial activity against *S. aureus* (Li et al., 2012; Lopez-Lazaro, 2009). Another study showed that luteolin reduces the production of α-toxin of *S. aureus* (Qiu et al., 2011).

The antibacterial activity of *I. viscosa* is largely believed to be due to high concentrations of the sesquiterpenes, occurring in the leaf extracts (Cafarchia et al., 2002). Sesquiterpenes were shown to possess antimicrobial activity against Gram positive bacteria such as *Bacillus subtilis*, *Escherichia coli* and *S. aureus* (Aljancic et al., 1999; Anke & Sterner, 1991; Fortuna et al., 2011; Lin et al., 2003). Additionally, sesquiterpenes lactones were reported to posses anti *H. pylori* activity (Konstantinopoulou et al., 2003). Previous studies have shown that the extracts from *I. viscosa* are effective for the treatment of digestive disorders, which could be related to their activity against clinical isolates of *H. pylori* and *C. albicans* (Ali-Shtayeh et al., 1998; Stamatis et al., 2003). Thus, the results of the current study are in accordance with previous studies and indicate that Jordanian medicinal plants might be a valuable source of novel antibacterial agents for *H. pylori*.

The results of this study indicate that most of the tested ethanol extracts induced cytotoxicity against both the clinical isolates of *H. pylori*. Moderate activities were shown for extracts of *P. incarnata*, *P. argentea*, *A. inculata*, *V. iphionoids*, *I. viscose*, *S. spinosum*, *P. guajava*, *L. officinalis*, *A. triphylla*, *O. syriaca*, *L. sativum*, *U. urens*, *A. graveolens and S. indicum*. These results are in correlation with previous reports about the general antibacterial

properties of some of these extracts or related plant extracts (Masadeh et al., 2013; Masoudi et al., 2012; Tomczyk et al., 2008).

Current results show the weak activity of ethanol extracts of *C. aronia* and *C. spinosa* against clinical isolates of *H. pylori*. These results are in accordance with previous results showing no antibacterial activity of these compounds against a panel of Gram positive and Gram negative bacteria (Masadeh et al., 2013). Yet, this lack of activity against *H. pylori* may be due to the method of preparing extracts including the choice of solvent. When a number of compounds are acting synergistically to produce a particular therapeutic effect, their separation may lead to loss of the desired activity. Moreover, low susceptibility of *H. pylori* clinical isolates among the tested plants does not necessarily imply their inefficacy *in vivo*. Bever (1986) and Garcia and co-workers (2003) had demonstrated immuno-modulation of chemical compounds from medicinal plants, many of which had been proven to be inactive or weakly active *in vitro* against pathogens. Also, as with some drugs, some of these plants may be more potent *in vivo* due to metabolic transformation of their components into highly active intermediates.

Conclusions

The results indicate that extracts from medicinal plants of Jordan namely, *A. triphylla* and *I. viscose*, might be a valuable source for the synthesis of new antibacterial drugs acting against *H. pylori*.

Declaration of interest

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