

**HELICOBACTER PYLORI GROWTH AND UREASE
INHIBITION BY CRANBERRY (*VACCINIUM
MACROCARPON*) EXTRACT: A PILOT STUDY**

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Abstract

The aim of the study was to evaluate the activity of three cranberry (*Vaccinium macrocarpon*) ethanol solutions on *Helicobacter pylori* growth and urease activity. We included numerous clinical *Helicobacter pylori* isolates and three methods: agar well diffusion method (AWDM), disk diffusion method (DDM) and urease inhibition test (UIT). The results were expressed as differences in inhibitory zone diameters (AWDM and DDM) or urease inhibition duration (UIT) by cranberry solutions compared to the ethanol control. AWDM showed that 400, 40 and 4 mg/l cranberry extracts inhibited the growth of 82.1, 57.1 and 42.8% of the isolates, respectively, while DDM at the highest cranberry concentration suppressed only 39.3% of the isolates. At 400, 40 and 4 mg/l, cranberry extracts also inhibited urease activity of 63.6, 54.5 and 40.9% isolates within 10 min, but of fewer isolates (18.2, 13.6 and 9.1%, respectively) at the 45th min. Although cranberry activity was dose- and strain-dependent, it affected more than half of the isolates at the two highest concentrations. DDM was less effective in detecting this activity. The cranberry extracts also inhibited the urease activity of *H. pylori*; however, in most cases, the inhibition was only temporary. Briefly, the high cranberry activity against *H. pylori*, together with its anti-adhesive, antioxidant, anti-biofilm and anti-cancer properties, justifies

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its use for prophylaxis or adjunctive treatment of chronic *H. pylori* infection. Importantly, UIT results suggest the benefit of regular cranberry intake over random intake.

Key words: *Helicobacter pylori*, growth, urease, inhibition, cranberry, *Vaccinium macrocarpon*

Introduction. Both European (*Vaccinium oxycoccus*) and American (*Vaccinium macrocarpon*) cranberry species are abundant in polyphenols, including anthocyanins, phenolic acids, flavonoids, dietary fibres, and vitamins, and are among the fruits that are rich in proanthocyanidins [1]. That is why cranberry fruits have many health-promoting benefits such as antioxidant, anti-biofilm, cancer-preventing, cardiometabolic, anti-atherosclerotic and antimicrobial activities [1].

Helicobacter pylori is one of the most common causative agents of chronic bacterial infections worldwide, with a global prevalence of 43.1% in the period of 2011 to 2022 [2]. The bacteria have a carcinogenic potential for some of the infected subjects, which is why cranberry's activities against *H. pylori* are of interest. There is also clinical evidence of beneficial properties of these fruits or their extracts, such as antibacterial, antiadhesive, and anti-inflammatory effects and improvement of antibiotic activity in *H. pylori* eradication regimens [3]. Cranberry extracts are considered safe overall, although in rare cases high doses can cause diarrhea or gastrointestinal discomfort [4]. Therefore, cranberry consumption may represent an additional dietary means of controlling *H. pylori* infections.

The aim of the present study was to investigate the action of *Vaccinium macrocarpon* fruit extract concentrate, especially at low concentrations, against the growth and urease activity of clinical *H. pylori* isolates by three methods. Since in some studies only single, or reference strains or only selected constituents of the cranberry fruits were investigated [5–8], in the present work, we included multiple clinical *H. pylori* isolates to determine strain-dependent differences in the effects of cranberry extracts.

Material and methods. Twenty-eight isolates from 28 patients (12 women, 15 men, and one boy) with gastroduodenal diseases were randomly selected from our laboratory collection and used in the study. The patients were 27 adults aged 32 to 89 years and one child aged 7 years. Twenty-seven patients had gastritis, one had gastroesophageal reflux disease (GERD) and one patient had had a pyloric ulcer. *H. pylori* isolation and identification was performed as described in our previous study [9].

We purchased CranRich cranberry (Natural Factors, Coquitlam, BC, Canada V3K 6Y2), of *Vaccinium macrocarpon* fruit extract concentrate 36:1 (1 g of extract containing 36 g of cranberry fruits) without added sugar, water, flavour, colour, or preservatives. We dissolved the substance in ethanol (96%) to obtain ethanolic cranberry extracts in concentrations of 400 mg/l, 40 mg/l and 4 mg/l cranberry. The cranberry extracts were sterilized by syringe filters of 0.22 µm pore size. The

methods used were the agar well diffusion method (AWDM), disk diffusion method (DDM) and urease inhibition test (UIT). The first two methods were carried out according to our prior publication [10] with some modifications.

For the AWDM, *H. pylori* inocula (matching McFarland 1 turbidity standard) were prepared in Mueller–Hinton broth (National Centre of Infectious and Parasitic Diseases, Bulgaria, NCIPD) and were plated onto Mueller–Hinton agar with 5% sheep blood in three directions by sterile swabs. Wells (8 mm diameter) were punched in the agar plates with a sterile stainless-steel borer. The wells were filled with 80 µl of the extracts or 80 µl 96% ethanol per well. The plates were incubated in a microaerophilic atmosphere with 5% O₂, 10% CO₂, and 85% N₂ (Campygen, Oxoid, Basingstoke, UK) at 37 °C for 48–72 h. Inhibitory zone diameters were measured in millimeters.

DDM was performed by sterile paper discs (6 mm diameter) soaked with 5 µl of either the cranberry solutions of 400 mg/l or 96% ethanol. The disks were allowed to dry and then used in the study. *H. pylori* isolates were suspended in Mueller–Hinton broth (adjusted to McFarland 1). Suspensions were spread onto the plates with sterile cotton swabs and thereafter the discs were added. The plates were incubated as above. The diameters of the inhibitory zones were measured in millimeters.

To avoid any potential errors when reading smaller zones, only inhibitory zones that measured ≥ 3 mm were considered for growth inhibition when reporting AWDM and DDM results.

For UIT, 1 ml of Christensen's broth (NCIPD), containing 2% urea per tube was used. Three cranberry solutions (15 µl) of ethanol are added to the tubes. Thereafter, 15 µl of *H. pylori* suspensions (McFarland 1) were added to each tube. Differences in urease test positivity were read after 10 and 45 min at room temperature.

AWDM, DDM and UIT were carried out on 28, 28 and 22 isolates, respectively. Table 1 presents the differences in inhibitory zone diameters between the alcohol control and cranberry solutions tested by AWDM and DDM. Table 2 shows the differences in the duration of urease inhibition by the cranberry solutions compared to the alcohol control.

A total of 256 combinations were made between the *H. pylori* isolates and the cranberry solutions or the 96% ethanol control. All experiments were run in duplicate.

Statistical analysis. Statistical analysis was carried out by Chi-square test with Fisher's exact test.

Results. By AWDM, the ethanolic extracts of 400, 40 and 4 mg/l cranberry, corresponding to 32, 3.2 and 0.32 µg/well, inhibited *H. pylori* growth of 23 (82.1%), 16 (57.1%) and 12 (42.8%) isolates with a mean increase of inhibition over the ethanolic control of 7.4, 4.2 and 1.8 mm, respectively. The difference in the number of inhibited strains between the cranberry concentrations of 400 mg/l

T a b l e 1

Increase in activity of three cranberry solutions in comparison with 96% ethanol control on 28 *H. pylori* strains by agar well diffusion method* (AWDM) and disk diffusion** (DDM) method

Method/details	AWDM*	AWDM*	AWDM*	DDM**
Cranberry solution (mg/l)	400	40	4	400
Amount of cranberry solution (μl)	80	80	80	5
Cranberry amount (μg) per well/disk	32	3.2	0.32	2
Mean increase (mm over control)	7.4	4.2	1.8	2.8
Range of increase (mm over control)	0–24	0–17	0–17	0–9
No. of inhibited strains	23	16	12	11
% of inhibited strains	82.1	57.1	42.8	39.3

AWDM and DDM results were reported for growth inhibition when the inhibitory zones were ≥ 3 mm. For both AWDM and DDM, only the inhibitory zones of 3 mm or more were considered to avoid error.

*By AWDM, the diameter of the wells was 8 mm and the mean inhibition by 96% ethanol was 10.5 mm, range of 8–20 mm.

**By DDM, the diameter of the disks was 6 mm and the mean inhibition by 96% ethanol was 6.4 mm, range of 6–10 mm.

T a b l e 2

H. pylori urease inhibition of 22 *H. pylori* strains by three cranberry solutions and 96% ethanol control

Cranberry solution	400 mg/l	40 mg/l	4 mg/l	96% ethanol
Cranberry amount (μg/tube)	0.4	0.04	0.004	0.0
No. of strains inhibiting urease at the 10th minute	14	12	9	0
% of strains inhibiting urease at the 10th minute	63.6	54.5	40.9	0.0
No. of strains inhibiting urease at the 45th minute	4	3	2	0
% of strains inhibiting urease at the 45th minute	18.2	13.6	9.1	0.0

and 4 mg/l was statistically significant ($p = 0.005$).

By DDM, 400 mg/l cranberry solution led to inhibition of 11 (39.3%) of the 22 isolates tested with a mean increase of inhibition over the ethanolic control of 2.8 mm. AWDM with 400 mg/l cranberry extract was better for detecting growth inhibition of *H. pylori* than DDM with the same concentration ($p = 0.002$).

By UIT, the cranberry extracts of 400, 40 and 4 mg/l cranberry inhibited *H. pylori* urease activity of 14, 12 and 9 isolates at the 10th minute and 4, 3 and 2 isolates at the 30th minute.

Discussion. Solvents of cranberry powder are acetone, methanol, ethanol, dimethyl sulphoxide (DMSO), or water. We chose to use ethanol as a solvent since the anthocyanin amount detected in 96% ethanol extract of cranberry powder was 18 to 34% higher than the amount in aqueous solution with 0.1 N hydrochloric acid in the study of ŠEDBARĚ et al. [11].

In the study of DAS et al. [12], minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic cranberry pomace extract against *Salmonella enterica* serovar Typhimurium, Enteritidis, and Heidelberg isolates were 8 and 16 mg/mL, respectively. However, in the study of SÁNCHEZ et al. [7], MICs of methanolic cranberry extract against anaerobic periodontal pathogens were much lower (0.10 mg/mL against *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* and 0.50 mg/mL against *Veillonella parvula*). Therefore, antibacterial activity of the cranberry shows large variations according to the bacterial species studied.

In the study of MATSUSHIMA et al. [5], hot water cranberry extract at 3.3 mg/mL almost fully inhibited *H. pylori* growth of two reference strains (NCTC11637 and 11638) in Brain-heart-infusion (BHI) broth and agar (BHI with 7% horse blood plasma agar) plates.

Strain difference in the effect of total cranberry extract on numerous *H. pylori* isolates has seldom been elucidated [13]. In the study of MATSUSHIMA et al. [13], the activity of cranberry extract powder produced as a mixture of cranberry fruits with hot water was evaluated against 27 clinical isolates. The authors used BHI with 7% horse blood plasma agar and detected susceptibility to < 2 mg/ml of the extracts in only 22.2% of the isolates [13].

In the present study, we confirmed the observation of Matsushima et al. [13] regarding the strain-specific inhibition of *H. pylori* growth by cranberry. However, in the present work, we used ethanol extracts of cranberry and two different methods (AWDM and DDM). Moreover, we compared all the results of inhibition by the cranberry extract with the ethanol used in parallel as a control. We found that most (> 82%) *H. pylori* isolates were inhibited by 400 mg/l cranberry solution, growth of more than half (57.1%) of the isolates was suppressed by 40 mg/l solution and > 42% of the isolates were inhibited even by 4 mg/l solution of the fruits. The inhibition was both strain-dependent and dose-dependent. The strain-dependent inhibition could be due to growth phase, metabolic, or other strain differences among the isolates.

H. pylori eradication is curative; however, eradication success has been decreasing in many countries, mainly due to clarithromycin, metronidazole, and levofloxacin resistance [14]. Therefore, the addition of non-antibiotic agents to eradication regimens has been investigated. LI et al. [15] found that encapsulated *V. macrocarpon* powder (280 mg) did not significantly improve *H. pylori* eradication. However, SEYYEDMAJIDI et al. [16] compared *H. pylori* eradication rates with a 14-day standard triple regimen (of lansoprazole, amoxicillin and clar-

ithromycin) and the same regimen plus an addition of 500 mg cranberry capsules twice daily (Liver Company, Canada). The authors observed a significantly higher eradication success (89%) in the patients treated with the addition of cranberry capsules than in those treated without the supplement (74%) [16]. Therefore, the dose and duration of cranberry extracts should be further investigated.

Urease is one of the virulence factors of *H. pylori*, which protects the bacteria from exposure to gastric acid and releases toxic ammonia [17]. An interesting finding in the present study was that within 10 min, the urease activity of *H. pylori* was inhibited in over about 2/3 (63.6%) of the isolates by the highest cranberry concentration (0.4 µg/tube) and in > 40% of the isolates even by the lowest concentration of 0.004 µg/tube. However, the inhibition was temporary, since within 45 min, the number of isolates with urease inhibition was only 18.2%, 13.6% and 9.1% by the two cranberry concentrations of 0.4, 0.04 and 0.04 µg/tube, respectively. Temporary *H. pylori* urease inhibition has been reported for other plants such as *Paeonia lactiflora* and *Parthenium hysterophorus* or their compounds [18,19]. However, to the best of our knowledge, this study reports for the first time the temporary urease inhibition of *H. pylori* by cranberry extracts as compared to control.

GOTTELAND et al. [20] used ¹³C-urea breath test (UBT) for children with negative UBT after a month of the prior testing. The authors found that as many as 80% of *H. pylori* isolates were only temporarily inhibited by the presence of cranberry juice, thus the use of cranberry and other non-antibiotic agents may be beneficial through regular intake rather than one-time consumption.

A limitation of the pilot study is that IUT was only performed on the last 22 isolates, since we wanted to include one more test method. Furthermore, as the work was a pilot study in nature, a larger number of clinical isolates could subsequently be tested, and the exact minimal inhibitory and minimal bactericidal concentrations determined. However, an advantage of this study is that numerous clinical isolates were used in AWDM and DDM and that the temporary inhibition of urease activity of *H. pylori* by UIT was detected.

Conclusion. In brief, cranberry activity against *H. pylori*, in addition to its antioxidant, anti-biofilm, antiadhesive and cancer-preventing properties, support the use of the non-antibiotic agent for the prevention or, as a supplement, for the treatment of chronic *H. pylori* infection. Although the activity of cranberry extracts was strain-dependent, the growth of > 42 to > 82% of the isolates was inhibited by the three cranberry solutions by AWDM. DDM was not as efficient as AWDM for determining cranberry inhibition of *H. pylori*. Importantly, UIT showed that urease inhibition of *H. pylori* by *V. macrocarpon* was only temporary, implying that regular cranberry consumption can show advantages over consumption at random.

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