

Effect on cell surface hydrophobicity and susceptibility of *Helicobacter pylori* to medicinal plant extracts

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Abstract

Effects of aqueous extracts of medicinal plants on ten *Helicobacter pylori* strains were studied by the salt aggregation test to determine the possibility to modulate their cell surface hydrophobicity and by an agar diffusion assay for detection of antimicrobial activity. It was established that aqueous extracts of bearberry and cowberry leaves enhance cell aggregation of all *H. pylori* strains tested by the salt aggregation test, and the extract of bearberry possessed a remarkable bacteriostatic activity. Pure tannic acid showed a result similar to that of bearberry and cowberry extracts which contained a large amount of tannins. In contrast, extracts of wild camomile and pineapple-weed, which blocked aggregation of *H. pylori*, contained small amounts of tannins and did not reveal any antimicrobial activity. Tannic acid seems to be the component of bearberry and cowberry aqueous extracts with the highest activity to decrease cell surface hydrophobicity as well as in antibacterial activity against *H. pylori*. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Specific lectin-like as well as non-specific charge and hydrophobic interactions seem to be commonly involved in pro-eucaryotic cell interactions [1]. Microbial adhesion to eucaryotic cells is often the first stage in many infections [2]. Pathogenic micro-organisms attach commonly to target tissues by specific adhesin-receptor mechanisms. However, microbial cell surface hydrophobicity (CSH) is often also asso-

ciated with binding to the specific cell and tissue receptors of mucosal surfaces in the infected host [3].

Previously, we have shown that aqueous extracts of some medicinal herbs, such as bearberry and cowberry, may enhance cell aggregation of *Escherichia coli* and *Acinetobacter baumannii*, whereas extracts of other herbs, such as wild camomile and pineapple-weed, attenuate it [4]. Now we have investigated another Gram-negative pathogen, *Helicobacter pylori*, a spiral-shaped bacterium causing active chronic type B gastritis and associated with peptic ulcer disease as well as gastric cancer [5].

H. pylori is sensitive to several antimicrobial

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agents in vitro, but the eradication of infection in vivo by using a single drug therapy is not effective [5]. Therefore, alternatives to conventional antibiotic therapy are now explored to try to intervene in early phase of *H. pylori* infection, including the first steps of adhesion to the gastric mucus layer and epithelial cells.

The aim of our study was to investigate the effect of aqueous extracts of bearberry, cowberry, wild camomile and pineapple-weed, widely distributed herbs in countries around the Baltic sea, on *H. pylori* surface properties and to establish the possible antibacterial action of these herbs. Some of these herbs are used mainly in traditional medicine for the prevention and treatment of various infectious and inflammatory diseases [6].

2. Materials and methods

2.1. Bacterial strains

H. pylori strain 17874 was obtained from the Culture Collection of the University of Gothenburg (CCUG, Sweden). Other *H. pylori* strains used (6, 33, 32, 253 and 12225) were clinical isolates from the collection in Lund [7]. In addition, fresh clinical isolates from the University Hospital in Lund were used (104/96, 113/96, 117/96 and 125/96). The strains were cultured on GAB-Camp agar (Difco) with defibrinated horse blood (5% per volume) [8].

2.2. Plant materials

The flowers of wild camomile (*Matricaria recutita* L., Asteraceae) and pineapple-weed (*Matricaria matricarioides* (Less.) Port, Asteraceae), and the leaves of bearberry (*Arctostaphylos uva-ursi* (L.) Spreng., Ericaceae) and cowberry (*Vaccinium vitis-idaea* L., Ericaceae) were collected in Estonia in 1996. The quality of all these medicinal herbs met the requirements of the European and USSR pharmacopoeias [9,10].

2.3. Preparation and analysis of aqueous extracts

Aqueous extracts of medicinal herbs were prepared as decoctions and infusions in proportion

1:10; decoctions were prepared from the leaves of bearberry and cowberry (extraction for 30 min at 100°C) followed by hot-filtering; and infusion from the flowers of wild camomile and pineapple-weed (extraction for 15 min at 100°C) followed by filtering after cooling at room temperature for 45 min [10]. The pH values of aqueous extracts of bearberry, cowberry, wild camomile and pineapple-weed were 4.7, 4.9, 5.2 and 5.4, respectively. The amount of extracted water-soluble substances and the content of tannins in aqueous extracts, were determined (mg ml^{-1}) using a titrimetric method [11].

2.4. Salt aggregation test (SAT)

For the determination of microbial cell surface hydrophobicity (CSH), the salt aggregation test (SAT) was used [12]. Bacterial cells from agar-grown cultures were washed twice with 0.01 M sodium phosphate buffer (pH 7.2) containing 0.15 M NaCl (PBS), re-suspended in the same buffer and adjusted to approximately 10^9 bacterial cells ml^{-1} (with an absorbance at 540 nm of approximately 1.0). Equal volumes (40 μl) of ammonium sulfate diluted in 0.02 M sodium phosphate buffer (pH 6.8) and bacterial suspensions were mixed in wells of a U-shaped microtitre plate. Incubation time for plates at room temperature was extended to 3 h, a modification of the standard procedure [12], to achieve visualisation of possible cell aggregation. SAT was defined as positive (+) if bacterial aggregation was clearly visible and negative (–) if no aggregation was observed; the concentrations of ammonium sulfate at which aggregation appeared was then registered. The SAT titre is defined as the lowest concentration of ammonium sulfate at which bacteria still show clearly visible cell aggregation. The strains were tested for auto-aggregation in sodium-phosphate buffer.

2.5. SAT assay with herb extracts

To affect the aggregation activity of *H. pylori*, 180 μl of bacterial suspension (10^9 cells ml^{-1}) were mixed with 180 μl of aqueous herb extract, or with 1% solution of tannic acid and left at room temperature for 15 min. Thereafter, 40 μl of this mixture was added to 40 μl of 0.05–3.0 M ammonium sulfate in phosphate buffer in the wells of an U-shaped micro-

Table 1
Constituents of aqueous extracts of four medicinal plants (mg ml⁻¹)

Constituents	Bearberry	Cowberry	Wild camomile	Pineapple-weed
Extractable substances	38.5	30.2	18.2	20.1
From that: tannins	19.2	7.4	1.0	1.2

titre plate. After incubation for 3 h at room temperature, microbial aggregation was estimated visually as described above.

2.6. Diffusion assay in GAB-Camp agar

Fresh *H. pylori* cultures grown on GAB-Camp agar for 3 days were harvested and suspended in 1 ml of sterile sodium-phosphate buffer. The density of the inoculum was adjusted to approximately 10⁹ bacterial cells ml⁻¹. The fresh GAB-Camp agar plate was flooded with 0.3 ml of the prepared inoculum and allowed to dry for 10 min. In the centre of the plate, wells were prepared into which 0.08 ml of the aqueous extract from various herbs or a solution of tannic acid was added and the results were read 6 days later after incubation by 37°C.

3. Results

The content of extracted substances in decoctions was approximately the same for both bearberry and cowberry and was similar in infusions of wild camomile and pineapple-weed (Table 1). Tannins constituted approximately 50% of all extractive substances in the aqueous extract of bearberry and approxi-

mately 25% in that of cowberry. However, in aqueous extracts of wild camomile and pineapple-weed, the tannins content was less than 6%.

For the determination of the CSH of different strains of the *H. pylori*, the SAT was used. None of the tested *H. pylori* strains aggregated either at or below 1 M ammonium sulfate, or in case of sodium-phosphate buffer; for five strains, aggregation was estimated at 3.0 M ammonium sulfate, and for five strains at 1.5 M (Fig. 1).

Decoctions of bearberry and cowberry as well as tannic acid solution enhanced cell aggregation of *H. pylori* while the activity of cowberry was stronger than that of bearberry, since cells of all tested strains aggregated after incubation with cowberry with 0.25 M ammonium sulfate concentration. The effect of a 1% w/v tannic acid (Sigma, St. Louis, MO, USA) was quite similar to that of bearberry and cowberry extracts. Infusions of wild camomile and pineapple-weed inhibited cell aggregation and the blocking activity of the aqueous extract of pineapple-weed was much stronger than wild camomile. At a high (3 M) concentration of ammonium sulfate, none of the *H. pylori* strains showed aggregation after incubation with pineapple-weed extract (Fig. 1).

The aqueous extract of bearberry possessed a remarkable bacteriostatic activity, whereas the decoctions of cowberry and of tannic acid solutions only weakly inhibited *H. pylori* growth (Table 2). No antibacterial activity against *H. pylori* was observed for aqueous extracts of wild camomile and pineapple-weed.

4. Discussion

This study demonstrates that specific medicinal plant extracts affect CSH of various *H. pylori* strains. Depending on the herbs used, cell aggregation activity can be enhanced or inhibited. We found, similarly to previous studies [13], that *H. pylori* does not re-

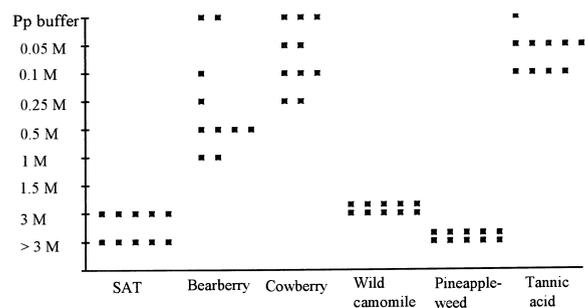


Fig. 1. SAT assays of *Helicobacter pylori* strains ($n=10$) with tannic acid and with various herb extracts. (The highest titres of SAT at which aggregation was detected or strains proved non-aggregative.)

Table 2

In vitro antibacterial activity of four medicinal plant extracts and tannic acid against *Helicobacter pylori* in an agar diffusion assay

Strains of <i>Helicobacter pylori</i>	Zone of inhibition (mm)				
	Bearberry	Cowberry	Wild camomile	Pineapple-weed	Tannic acid
33	33 (± 8)	17 (± 1)	0	10 (± 0)	16
6	32 (± 5)	12 (± 0)	10 (± 1)	10 (± 1)	25
32	28 (± 3)	12 (± 2)	0	0	15
17874	42	12	0	0	16
253	37 (± 7)	16 (± 5)	11 (± 1)	11 (± 1)	n.d.

n.d., test was not carried out.

veal high CSH in the salt aggregation test. However, some strains of *H. pylori* have been reported to express high CSH, as measured by hydrophobic interaction chromatography [14]. Since we detected several differences in SAT values, the individual strains of *H. pylori* may possess various degrees of CSH.

For many bacterial infections, different pathogenesis phases can be distinguished, such as mucosal surface adhesion, tissue colonisation and subsequent invasion [1,15]. Adhesive strains often possess high CSH as determined by SAT and other methods [16]. High CSH appears to be important in the pathogenesis of several micro-organisms associated with gastrointestinal infections [15]. We suppose that a low CSH of *H. pylori* may allow this pathogen to attach and penetrate the gastric mucus layer. The application of medicinal plant extracts in modulation of this first mucosal surface contact phase of infection by enhancing or lowering CSH values would be important for treatment and prophylaxis in modern medicine.

Previously, it has been shown that it is possible to affect CSH and tissue adhesive properties of some bacterial pathogens with sub-lethal doses of antibiotics [17]. However, antibiotic therapy can cause problems with the development of antibiotic resistant *H. pylori* strains [18], but the combined therapy with medicinal plant extracts may help to postpone the issue.

Medicinal plants serve as a useful source of novel drugs [19]. Plants that are widely used in folk medicine, such as bearberry, cowberry, wild camomile and pineapple-weed, were chosen for this study. The leaves of bearberry and cowberry contain mainly hydroquinone derivatives (arbutin, methylarbutin), tannin, flavonoids, triterpenes and phenol-carboxylic acids and the flowers of wild camomile

and pineapple-weed contain mainly essential oil, flavonoids, sesquiterpene lactones, coumarins and mucilage [6]. We found that bearberry and cowberry were the only tested herbs expressing some antimicrobial activity against *H. pylori*. However, several studies have shown that the anti-microbial activity of these medicinal plants is too low to kill microbes or to inhibit their growth in animal tissues, their actions must be based on other mechanisms [20].

In this study, we have shown that CSH of *H. pylori*, as measured by SAT, can be modulated by medicinal plant extracts. We have found that aqueous extracts of bearberry and cowberry enhance CSH, whereas aqueous extracts of wild camomile and pineapple-weed inhibit cell aggregation of all *H. pylori* strains tested in SAT. The CSH-enhancing or -lowering activity of the drugs was similar for *E. coli* and *Acinetobacter baumannii* [4] and all *H. pylori* (Fig. 1) strains tested.

To explain which compounds of the water extracts of medicinal plants interfere with CSH of *H. pylori*, we determined the total amount of both water-extractable substances and tannins. Tannins comprise a large group of complex substances that are widely distributed in the plant kingdom and almost every family of plants contain species which contain tannins [21]. The property of tannins to precipitate proteins from solution; when applied to living tissues. Although some tannins may appear to be glycosidic by nature, the majority of them are probably not, and when hydrolysed, yield relatively simple phenols. Such phenolic groups are responsible for tissue astringent and antiseptic actions [21]. According to our data, bearberry and cowberry leaves contain significantly more tannins compared to wild camomile and pineapple-weed flowers and a high content of tannins in these herbs was reported previously [22]. It is

noteworthy that pure tannic acid, as tested by SAT, gave similar results as bearberry and cowberry extracts, suggesting that tannic acid may be the component of the herb extracts with the strongest effect in relation to its effect on CSH of *H. pylori*. Furthermore, the antibacterial activity of bearberry can be accounted for by tannic acid, as the antibacterial activity of the solution of tannic acid is similar to that of bearberry extracts. The aqueous extract of cowberry showed a weak antimicrobial activity, which correlates well with the smaller content of tannic acids (approximately 25% versus 50%, in the case of bearberry).

In contrast, water extracts of wild camomile and pineapple-weed with very low content of tannic acids blocked aggregation of *H. pylori*. The cell aggregation blocking compounds of these herbs are difficult to define.

This study demonstrated that: (1) the substances in aqueous extracts of bearberry and cowberry are able to enhance *H. pylori* cell aggregation and antibacterial activity; and (2) the aqueous extracts of wild camomile and pineapple-weed block cell aggregation of *H. pylori* and do not express any antibacterial activity. A possible application of these findings in the pharmaceutical industry for the production of compounds supporting antibiotics for treating *H. pylori* infection seems to be worth exploring.

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