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# Aqueous extracts and polysaccharides from Liquorice roots (*Glycyrrhiza glabra* L.) inhibit adhesion of *Helicobacter pylori* to human gastric mucosa

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ABSTRACT

*Aims:* Aqueous extracts from the roots of *Glycyrrhiza glabra* L. (Fabaceae) are widely used for treatment of stomach ulcer. The clinical proven effects are related to the presence of anti-inflammatory 12-keto-triterpensaponins in the extracts. Apart from that the influence of *Glycyrrhiza glabra* extract on the bacterial adhesion of *Helicobacter pylori* to stomach tissue was to be investigated. Additionally the influence of *Glycyrrhiza glabra* secondary compounds on the bacterial adhesion of *Porphyromonas gingivalis*, a major pathogen for induction of periodontal inflammations was to be investigated.
*Methodology: In vitro* cytotoxicity against *Helicobacter pylori* was investigated by agar diffusion assay; antiadhesive properties of aqueous extract, raw polysaccharides and purified polysaccharide fractions was investigated by means of an *in situ* adhesion assay with FITC-labelled bacteria on tissue slides of human stomach resectates.
*Results:* Aqueous extract (1 mg/mL) of *Glycyrrhiza glabra* significantly inhibited the adhesion of *Helicobacter pylori* to human stomach tissue. This effect was related to the polysaccharides isolated from the extract, with one purified acidic fraction (0.25 SPB) as main active polymer. Purified polysaccharides did not exhibit direct cytotoxic effects against *Helicobacter pylori* and did not influence hemagglutination. Additionally raw polysaccharides from *Glycyrrhiza glabra* were shown to have strong antiadhesive effects against *Porphyromonas gingivalis*.
*Conclusion:* Aqueous extracts and polysaccharides from the roots of *Glycyrrhiza glabra* are strong antiadhesive systems, which may be used as potent tools for a further development of cytoprotective preparations with anti-infectious potential.

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## 1. Introduction

Liquorice root from *Glycyrrhzia glabra* L., also named as sweet root is a ligneous perennial shrub growing in the Mediterranean region, Asia Minor and Middle East and is also cultivated widely in southern Russia. The medical use of Liquorice has a long history and since more than 2000 years the preparation and application of manifold liquorice extracts, mainly based on an aqueous extraction, is well documented (Ody, 2000). Traditional uses of liquorice are mainly in the treatment of peptic ulcer and as expectorant for cough and bronchitis. For review on traditional uses and pharmacological profiles of liquorice and its bioactive compounds see Nassiri and Hosseinzadeh (2008). Modern review of phytochemical investigations, clinical particulars and pharmacological properties gave evidence that the use of liquorice is justified and an ESCOP monograph gives therapeutic indications for adjuvant therapy of gastric/duodenal ulcers and gastritis as well as expectorant for coughs and bronchial catarrh (ESCOP, 2003). From the phytochemical point of view the root is characterized by the typical 12-keto-triterpensaponins with glycyrrhizin as lead compound and generally regarded to have anti-inflammatory potential by inhibition of a 11β-hydroxysteroid dehydrogenase (Monder et al., 1989). Other typical secondary compounds from liquorice root are flavonoids (liquritin, glabrol), isoflavones (glabrene, glabridin), chalcones (isoliquiritin), coumarines (liquocoumarin) and stilbenoides besides minor amounts of essential oil and polysaccharides.

When looking at the pharmacological mechanisms responsible for the antiulcer activity of liquorice extracts the anti-inflammatory activity of glycchyrizin and the spasmolytic effects of flavonoids and chalcones are regarded in general as the main principles. On the other side one of the main causes of stomach ulcer are chronic infections by *Helicobacter pylori*, a spiral, Gram-negative,

*Abbreviations:* AEC, anion exchange chromatography; AGP, arabinogalactan protein; HPAEC, high pressure anion exchange chromatography; RPS, raw polysaccharides; SPB, sodium phosphate buffer.

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microaerophilic bacterium which is known to colonize the human stomach. *Helicobacter pylori* adheres strongly to gastric epithelia or their superficial mucus layers. *Helicobacter pylori* is considered to promote the development of chronic gastritis, peptic ulceration, gastric carcinoma and gastric MALT-lymphoma (Warren and Marhall, 1983). Equipped with the enzyme urease, the microbe is enabled to survive gastric pH levels of ≥2.0 in the presence of urea. The bacterium is strongly dependent on the outer-cell wall adhesins which specifically interact with the corresponding counterparts on epithelia side, enabling the bacterium to bind physically and stable to the host cell epithelia. For review on the complex nature of these adhesins see Evans and Evans (2000) and Kusters et al. (2006). Strong efforts are made since several years to identify compounds with inhibitory activities on such adhesins. Antiadhesive compounds can inhibit the docking process of *Helicobacter pylori* to the stomach tissue, leading to a diminished incidence of infection. Because the most important adhesins of *Helicobacter pylori* are initiating carbohydrate–carbohydrate or protein–carbohydrate interactions, the blocking of these adhesins by exogenous carbohydrates seems promising. Within the following studies the hypothesis was to be investigated, if the potential antiulcer activity of liquorice root extracts may be also due to a direct cytotoxic activity or an indirect antiadhesive effect of aqueous extracts against *Helicobacter pylori*. When searching the respective literature evidence is found for a moderate direct cytotoxic effect of liquorice, glycyrrhizic acid and some flavonoids against *Helicobacter pylori* (Fukai et al., 2002; Krausse et al., 2004). Minor direct anti-Helicobacter effects were reported also for the *Glycyhrriza* species, *Glycyhrriza aspera* (Nariman et al., 2004). From that point of view it was to be investigated if beside the direct cytotoxic effects also indirect antiadhesive properties can be found for the respective liquorice extracts.

In order to answer this question an aqueous extract and the polysaccharides isolated from this extract were shown to be significantly antiadhesive against the bacterial docking process of *Helicobacter pylori* to human stomach tissue.

Further investigations of the active polysaccharides on other potential antiadhesive activities were performed against *Porphyromonas gingivalis*, a Gram-negative, anaerobic bacterium, widely considered as a major pathogen in the development of destructive periodontal diseases such as chronic or aggressive periodontitis. *Porphyromonas gingivalis* expresses a number of pathogenicity factors, including adhesins, LPS, capsule, collagenase, hemagglutinin, haemolysins and extracellular hydrolytic enzymes, especially proteinases. *Porphyromonas gingivalis* adheres to epithelial cells, red blood cells, collagen complexes and other bacteria, and has furthermore been found to invade host cells, e.g. epithelial cells (Sandros et al., 1993). The invasion and internalization of *Porphyromonas gingivalis* into tissue is normally started by a positive adhesion to certain surface factors of the target cells. The major adhesins are located on the tip of its fimbriae to attach the surface to epithelial cells. Previous studies clearly have shown that the respective adhesins recognizes glycosylated structures on epithelium side. Also gingipain peptides as secreted proteins are found on the bacterial surface with a strong adhesion capacity (Chen and Duncan, 2004).

At the moment no anti-infectious compounds are available to inhibit the destructive work of *Porphyromonas gingivalis*. Despite increased mouth hygiene the bacterium is not captured by standard mouth hygiene. *Porphyromonas gingivalis* can only get invasive when getting into a strong adhesive interaction with the epithelium. This means that products which inhibit this interaction can provide valuable tools for prophylaxis. Because the adhesion process is mainly mediated by carbohydrate–protein and protein–protein interactions the use of *Glycyrrhiza glabra* polysaccharides (which is not a traditional use of the herbal material) to block the adhesion process was worth to be investigated.

## 2. Materials and methods

### 2.1. General experimentation procedure

Liquorice root (batch 43419085) was purchased from Caesar & Loretz (Hilden, Germany). Identification, purity and content were determined according the respective methods of the European Pharmacopoeia (2008). Reference material of the root material is stored in the herbarium of the Institute of Pharmaceutical Biology and Phytochemistry. Okra fresh extract was prepared according to Lengsfeld et al. (2004). If not stated otherwise chemicals were purchased from VWR (Darmstadt, Germany) in analytical quality grade. Fluorescence microscopy was performed using a Zeiss Standard 16 microscope with fluorescence condensor IV FI and camera system MC 63.

### 2.2. Preparation of aqueous extract, RPS and AEC fractions

Pulverized root material (50 g) from *Glycyrrhiza glabra* was extracted at 8 °C with water (250 mL) for 20 h. The extract obtained after centrifugation (2400 × *g*, 15 min) was stored at −20 °C for further use for functional tests.

For preparation of RPS pulverized root material (200 g) from *Glycyrrhiza glabra* were defatted by Soxhlet extraction (ethylacetate, 6 h). The defatted and air-dried material (160 g) was extracted three times at 8 °C with water (800 mL) for 20 h. The extracts obtained after centrifugation (2400 × *g*, 15 min) were combined and concentrated under reduced pressure at a 40 °C not exceeding temperature. High molecular constituents were precipitated in the fourfold amount of ethanol. The precipitate was isolated by centrifugation (3000 × *g*), suspended in 20 mL of water, dialyzed (Cellulose membranes, MWCO 3500 Da, Roth, Karlsruhe, Germany) and lyophilized to yield 2.5% raw polysaccharides (RPS), related to the starting root material.

RPS was fractionated by AEC on DEAE Sephacel® column (30 cm × 2.5 cm) in the phosphate form and elution by a step gradient of deionized water, sodium phosphate buffers pH 6.0, ion strength 0.1, 0.25, 0.5, 1 mol/L, and 0.05N NaOH, flow 100 mL/h, fraction size 2 mL. Carbohydrate-containing fractions were pooled, concentrated under vacuum, dialyzed and lyophilized.

### 2.3. Carbohydrate analysis

Total carbohydrates in AEC- and GPC-fractions were assayed using the resorcinol–sulphuric acid test Monsigny et al. (1988). Determination of total uronic acids was performed according to the method of Blumenkrantz and Asboe-Hansen (1973) with *o*-hydroxydiphenyl in a modification for 96-well-microtiter plates using galacturonic acid as reference. Quantification of monomeric carbohydrates was accomplished on ion-exchange HPLC with pulsed-amperometric detection (Dionex, Idstein, Germany), Bio LC, with AS50 autosampler, GS50 gradient pump, AS50 oven and ED50 electrochemical detector on a CarboPac™ PA1, analytical column, 2 mm × 250 mm, CarboPac™ PA1, guard column 2 mm × 50 mm and BorateTrap™ Trap, 4 mm × 50 mm. Elution was done with gradient program using water and NaOH 0.1 M for the determination of neutral sugars, and a ternary gradient from water, NaOH 0.1 M and NaOAc 0.5 mM for quantification of uronic acids.

Polysaccharides were hydrolyzed with trifluoracetic acid 2 mol/L (121 °C, 1 h).

Determination of molecular weight distribution of polysaccharides was performed by FPLC (GE Healthcare, Freiburg, Germany) on a Superose®6 column using standard dextrans (Sigma, Deisenhofen, Germany) for calibration. Void volume was determined with DextranBlue® (Sigma, Deisenhofen, Germany). Quantification of residual protein was performed according to Bradford (1976).

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