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Antiadhesion as a functional concept for protection against uropathogenic *Escherichia coli*: *In vitro* studies with traditionally used plants with antiadhesive activity against uropathognic *Escherichia coli*

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ABSTRACT

Ethnopharmacological relevance: Investigation of medicinal plant extracts traditionally used against uncomplicated urinary tract infections (UTI) and identification of antiadhesive effects under *in vitro* conditions against binding of uropathogenic *Escherichia coli* (UPEC) on bladder cell surface.

Materials and methods: Literature search on traditionally used medicinal plants for UTI was performed by online data bases and standard herbal monographs. For further identification shortlisting was done by intensive evaluation of results by plausibility and phytochemical aspects. Plant material with documented antibacterial effects was not considered for further investigations. Direct cytotoxicity of EtOH–water (1:1; v/v) extracts of the shortlisted plants was investigated against UPEC strain 2980 and bladder cell line T24. Inhibition of UPEC adhesion to T24 cells was monitored either after pretreatment of bacteria or eukaryotic cells by flow cytometry.

Results: Literature search on traditionally used medicinal plants for UTI resulted in 275 plant species, from which 20 were shortlisted by a validated selection process for experimental testing. While direct cytotoxicity of the extracts (1–2000 μg/mL) against UPEC and T24 cells was excluded significant antiadhesive effects were monitored for five plant extracts. Two of them, prepared from the rhizome of *Agropyron repens* L. and the stigmata of *Zea mays* L. decreased bacterial adhesion (IC_{25} 630 μg/mL, IC_{50} 1040 μg/mL, resp.) by interacting with bacterial outer membrane proteins, which was shown by pretreatment of UPEC. Preparations of three plant extracts from the leaves of *Betula* spp. (according to European pharmacopoeia 7.0), *Orthosiphon stamineus* BENTH. and *Urtica* spp. showed antiadhesive effects by interacting with T24 cells (IC_{50} 415, 1330 μg/mL, resp. IC_{25} 580 μg/mL). Combination of two extracts, one interacting with the bacterial surface (*Zea mays* L., *Agropyron repens* L.) and one with the eukaryotic target (*Orthosiphon stamineus* BENTH.) revealed synergistic effects, as shown by strongly decreased IC_{50} values (131 μg/mL, 511 μg/mL, resp.).

Conclusions: Different plant extracts, traditionally used for UTI, exhibit antiadhesive effects against UPEC under *in vitro* conditions. Molecular targets can be different, either on the bacterial or on the host cell surface. Combination of these medicinal plants with different targets, as observed often in phytotherapy, results in synergistic effects.

1. Introduction

Uncomplicated urinary tract infections (UTI) with uropathogenic *Escherichia coli* are one of the most common infections world-wide. Within the exploration and assessment of therapeutic alternatives for the antibiotic standard treatment (for review see Sivivk and Mobley, 2010) the prophylaxis of a potential infection by antiadhesive compounds is getting more and more into the experimental and clinical research focus. An optimized antiadhesive entity should interact more or less specifically with adhesins of the pathogen, leading to a significant inhibition of the docking-process between pro- and eukaryotic cells (Wittschier et al., 2007a, 2009; Hensel et al., 2009; Niehues et al., 2011; Löhr et al., 2011; Gescher et al., 2011b). On the other hand also surface structures on the eukaryotic host cell, the so-called antigens, can be blocked certain antiadhesive; this leads to the effect that pathogens with adhesins complementary to these antigens will not recognize the host cell any more. In both cases invasion or infection of the epithelial cells should be minimized. A practical use of such antiadhesive entities can lead to prophylaxis for new infections and reduction of recurrence.

In case of UTI extraintestinal, uropathogenic *Escherichia coli* (UPEC) are known to be strongly adhesive and virulent by

Abbreviations: BCR, Bacteria-cell ratio; UPEC, Uropathogenic *Escherichia coli*; UTI, Uncomplicated urinary tract infections

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different surface factors, especially fimbriae. Such filamentous adhesive structures enable UPEC to adhere and to invade extremely effective to bladder cells. Disruption of the barrier during the course of an UTI can occur as a consequence of UPEC-induced exfoliation of infected bladder cells followed by strong inflammatory response (for review see Dhakal et al., 2008). This exfoliation again leads to colonization and long-term persistence of UPEC within the urinary tract with a high recurrence rate (Mulvey et al., 2001). UPEC strains are characterized by different fimbriae types which are responsible for the different pathogenic effects. Fimbriae from *Escherichia coli* are assembled from different adhesins, which attach to specific oligosaccharide receptors on the uroepithelial cells. Type 1 fimbriae are hemagglutinins with mannose-residues as complementary receptor structures. The adhesion, mediated by these structures can be blocked by mannose. The protein subunit, responsible for adhesion and internalization into the cell is named as fimH (Schembri et al., 2001). 50–70% of UTI are associated with such type 1-fimH associated *Escherichia coli* bacteria, 35–50% with papG.

P-fimbriae, encoded by the *pap* (pyelonephritis-associated pili) gene are interacting with P-antigens on erythrocytes and bladder cells (Eden and Leffler, 1980). These antigens are characterized by glycolipids with a typical Gal-(α1→4)-Gal-β-disaccharide sequence. The subunits at the distal end of P-fimbriae, responsible for the binding to the antigen are named as PapG and are subdivided into PapG class I, II and III (for review see Lane and Mobley, 2007). *Escherichia coli* with such P-fimbriae is mainly found in patients suffering from complicated pyelitis of the kidney and also sometimes during UTI (Johnson, 1991).

Additionally S-fimbriae and F1C-fimbriae-bearing *Escherichia coli* can be found as pathogens in UTI with S-fim binding to sialylgalactosyl residues (Parkkinen et al., 1989) and F1C-fim interacting with GalNac-(1→4)-Gal (Khan et al., 2000). S-fim bearing *Escherichia coli* are isolated from patients during severe sepsis and meningitis and only rarely from patients with UTI (Johnson, 1991); in contrast to that F1C-*Escherichia coli* are detected during about 14% of UTI (Pere et al., 1985).

Type 1 fimbriae associated adhesin FimH, Dr family adhesins and bacterial toxin CNF1 have been shown to trigger and/or modulate bacterial entry into host epithelial cells (Martinez et al., 2000; Selvarangan et al., 2000), often in combination with complement C3 opsonisation (Li et al., 2009).

During the search for antiadhesive compounds cranberry extracts (*Vaccinium macrocarpon* Ait.) are often associated with the prevention of UTI (Zafriri et al., 1989) and also a recent Cochrane review highlighted the positive evidence that cranberry juice can decrease the frequency of UTI (Jepson et al., 2004). Besides the clinical evidence that cranberry extracts are effective, Foo et al. (2000a, 2000b) reported that A-type proanthocyanidin (PCA) trimers from cranberry are responsible for an antiadhesive effect against P-fim *Escherichia coli*. This finding was based on a hemagglutination assay and an *in vitro* antiadhesion assay using *Escherichia coli* and its binding to P-receptor-coated beads with an immobilized [Gal-(1→4)-Gal]-disaccharide. The respective data have been reproduced (Howell et al., 2005; Turner et al., 2005) and inserted into the literature over several years (for review see Howell, 2007). On the other hand no registered cranberry-containing drug products are available on the market; cranberry-containing functional foods (or nutraceuticals) found in the market do not comply with scientific standards concerning purity or content of a standardized extracts. Therefore a strong need for identifying antiadhesive actives against UPEC for serious drug-development is obvious.

In traditional European phytomedicine aqueous decoctions or ethanol–water extracts from many herbal drugs are used frequently for UTI. For rationalized explanation of potential effects an increase in urinary flow, as well as spasmolytic and antibacterial activities are mentioned. During critical data review potential hyperosmolar effects are assessed to be very low and also antibacterial activities should not be over interpreted. On the other side improvement of clinical symptoms by these preparations is reported by many practical clinicians. For that reasons a testing on potential antiadhesive effects (Lengsfeld et al., 2007; Wittschier et al., 2007b; Löhr et al., 2011; Gescher et al., 2011a, 2011b) of these herbal materials seems scientifically attractive in order to obtain more insights into a potential mode of action. Therefore the following study reviews systematically the literature on traditionally used herbal drugs for UTI (literature available by the authors), followed by a selection of plant materials according phytochemical aspects and plausibility, followed by *in vitro* testing of the respective extracts on potential antiadhesive effects against UPEC.

2. Materials and methods

2.1. General experimentation procedure

If not stated otherwise all chemicals were purchased by VWR (Darmstadt, Germany). Herbal materials, sources, identification, and voucher sample IDs are listed in Table 1. Identification of plant materials was done according to the relevant monographs of European Pharmacopoeia (2008, 2011) and Hänsel et al. (1992).

2.2. Preparation of extracts for functional testing

10 g of freshly powdered plant material from the respective plant material (see Table 1) was extracted with 100 mL of EtOH–water (1:1 v/v) by rotor-stator homogenizer (Ultra-Turrax®, T25 IKA, Staufen, Germany, maximum speed, 10 min). The suspension was centrifuged for 10 min at 3000*g*. The supernatant was frozen at −80 °C and lyophilized. Yields obtained are listed in Table 1.

2.3. Uropathogenic Escherichia coli (UPEC) strain and growth conditions

UPEC strain 2980 (DSM 10791), identified by PCR by fimH, papG2, prsG3, focG and hlyA genes, was cultured at 37 °C on Loeb agar (per 1 L: 15 g agar, 10 g bacto-tryptone, 8 g sodium chloride, 1 g yeast extract, 1 g glucose) for 24 h. Plates were stored at 4 °C, followed by inoculation after two weeks and incubation overnight at 37 °C and storage again at 4 °C.

Bacteria were agar-grown for 24 h, harvested and used for inoculation of Loeb agar containing 0.2% $CaCl_2$. The cultures were grown overnight at 37 °C. Growth was monitored by optical density ($OD_{640\ nm}$) and bacteria in exponential phase were used for further experiments. Adhesin expression was routinely controlled by RT-PCR.

The same conditions were used for papG1, cnf-16 positive UPEC strain J96 (ATCC 70033).

Note: Other UPEC strains tested (e.g. J96) did also adhere to T24 cells but were shown to induce strong cell toxicity, probably by the protein products from the papG1, cnf-1 genes. Therefore validated adhesion studies could not be performed by using the virulent strains (e.g. J96) while the use of 2980 strain gave clear and reproducible results in adhesion testing.

2.4. Cell culture

T24 cell line (ATCC HTB-4), derived from human urinary bladder carcinoma (Bubenik et al., 1973) was kindly provided by Prof. Straube (University Jena, Germany). Cells were cultured in Dulbecco's modified Eagle medium(DMEM, high glucose with