

## Summary

Urinary tract infections (UTI) are one of the most common infectious diseases worldwide, in most cases caused by uropathogenic *E. coli* (UPEC). Impeding the bacterial adhesion to bladder epithelial cells is a promising target for an alternative strategy to conventional antibiotic treatment. Therefore, phytochemical and functional tests concerning the two traditionally used herbal drugs *Orthosiphonis folium* (*Orthosiphon stamineus* Benth.) and *Ononidis radix* (*Ononis spinosa* L.) were performed.

Within a flow cytometry-based adhesion assay an aqueous (OWE) and an acetone (OAE) extract of *Orthosiphon* leaves showed a significant and concentration dependent reduction of UPEC UTI89 adhesion to human T24 bladder cells, which was more pronounced for OAE than for OWE (reduction of 69 % at 75 µg/mL and 40 % at 2000 µg/mL). Cytotoxicity was not observed. A chromatographic comparison of the two extracts demonstrated a much higher concentration of polymethoxylated flavones in OAE than in OWE. Five of these flavones (5-hydroxy-6,7,3',4'-tetramethoxyflavon, salvigenin, 5,6,7,4'-tetramethoxyflavon, eupatorin and ladanein) were isolated by fast centrifugal partition chromatography and preparative HPLC and structurally characterized. Sinensetin, a flavone known from *O. stamineus* and four of the isolated flavones showed potent antiadhesive properties against UPEC UTI89 (inhibition of adhesion of up to 61 % at 200 µM). Only eupatorin could not provide a significant antiadhesive effect, which could be attributed to a hydroxylation of the B-ring. The relevance of this structural characteristic for antiadhesive activity of flavones was confirmed by analysis of the B-ring monohydroxylated flavones diosmetin and chrysoeriol (reduction of adhesion of 13 and 9 % at 200 µM). Continuative investigations constituted that a simultaneous presence of both cell types (bacteria and bladder cells) was necessary for an antiadhesive activity of sinensetin, while OWE and OAE also showed activity after preincubation of just one cell type. Furthermore, both extracts and sinensetin exhibited an antiinvasive activity, which was higher than the antiadhesive effects of sinensetin and OWE (reduction of invasion of 92 % at 200 µM and of 66 % at 2000 µg/mL). Within a gene expression analysis of UPEC using qRT-PCR OAE, but not sinensetin, showed a reduced expression of genes, which are encoding for adhesive pili. The results indicated a decreased biosynthesis of type 1-, P-, S- and F1C-pili as well as of curlis as underlying antiadhesive mode of action. During analysis of the influence of bacterial phenotypes no reduced expression of hemagglutinating pili (P-, S- and Dr-Pili) or curlis was observed for both extracts and sinensetin, while the ability to agglutinate yeast cells of type 1-

pili was reduced by OAE after 24 hours incubation. OWE and sinensetin did not cause such an effect. To investigate the transferrability of the *in vitro* data to *in vivo* conditions, a biomedical study was performed. Urine samples from 20 voluntary probands were collected before and after a seven-day intake of an aqueous infusion of Orthosiphon leaves (daily dosage 12 g) and analysed according to their antiadhesive properties. Compared to day 1-controlurine the mean bacterial adhesion expressed a 20 % decrease for day 3-urine, 8 % for day 6-urine and 17 % for day 8-urine. The reduction caused by urines, which were pooled from all probands, was more pronounced (reduction of adhesion of up to 48 % (pooled day 8-urine)). An additional analysis of subpopulations showed a stronger antiadhesive activity of urines from male probands than for female ones. Moreover, a strong, although not significant reduction of bacterial invasion was observed. A qRT-PCR of UPEC after incubation in pooled day 1- and day 8-urine showed an increase in the expression of the motility-associated gene *fliC*, which was already known for OWE under *in vitro* conditions. An influence on other genes was not observed. During phenotypical investigations an increase of motility as a potential reason for the antiadhesive activity was confirmed, while biosynthesis of type 1-pili and curlis was not influenced.

An aqueous extract of *O. spinosa* roots, which contained a high amount of isoflavonoids, showed a strong reduction of UPEC UTI89 adhesion to T24 bladder cells (reduction of bacterial adhesion of up to 69 % for 1000 µg/mL). A reduction of bacterial invasion of 69 % for 1000 µg/mL confirmed this antiadhesive effect, which was also observed after preincubation of bacteria and preincubation of bladder cells. The extract did not influence the biosynthesis of type 1-pili or curlis in a relevant manner.

The present data indicates pronounced antiadhesive activities *in vitro* for extracts from *O. stamineus* leaves and *O. spinosa* roots as well as for polymethoxylated flavones, which are due to a pleiotropic mode of action. Moreover, the antiadhesive effects were confirmed for an oral intake of an aqueous Orthosiphon leaves extract by a biomedical study and seemed to be a consequence of an increased bacterial motility. The results are rationalizing the use of both medicinal plants as phytotherapeutic agents in the treatment of UTI.