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II. SUMMARY

In the scope of this thesis the anti-adhesive and functional activity of an extract and isolated polyphenols from *Rumex acetosa* L. (Polygonaceae) against *Porphyromonas gingivalis* were investigated.

The proanthocyanidin-enriched acetone-water (7:3) extract RA1, obtained from the aerial parts of *R. acetosa* L., was shown to exhibit a significant anti-adhesive activity (coincubation protocol: IC_{50} = 10.8 µg/mL) against *P. gingivalis* (ATCC 33277) towards KB cells *in vitro*. Variation of incubation protocol revealed an interaction of the compounds from RA1 with bacterial surface structures (Preincubation of bacteria: 100 µg/mL: 30 % inhibition). Enhanced anti-adhesive activity, which has been shown in coincubation experiments of KB cells together with *P. gingivalis* and RA1 strongly indicates an additional interference in the interaction of bacteria with host cell receptors (10 µg/mL: 42 % inhibition). Structure-activity relationship of relevant polyphenols from RA1 gave evidence that flavan-3-ols and oligomeric proanthocyanidins are responsible for the inhibition of bacterial adhesion. Especially esterification of flavan-3-ols with gallic acid and trihydroxylation of the B-ring was shown to be essential for activity. Furthermore, the degree of oligomerization of these building blocks was correlated to an increase in activity: epicatechin-3-O-gallate (20 µM: 21 % inhibition); epicatechin-3-O-gallate-(4β->8)-epicatechin-3-O-gallate (20 µM: 61 % inhibition); epicatechin-3-O-gallate-(4β->8)-epicatechin-3-O-gallate-(4β->8)-epicatechin-3-O-gallate (10 µM: 99 % inhibition). Flow cytometric investigation on the effect of the adhesion of a clinical isolate of *P. gingivalis* to KB cells confirmed this anti-adhesive activity (10 µg/mL: 90 % inhibition).

To estimate the influence of saliva for a potential clinical use of the extract, it was demonstrated that porcine pancreas mucin does not neutralize the activity of RA1.

Additionally, RA1 in concentrations of ≥ 50 µg/mL was shown to impair the formation of an intact monocultural biofilm of *P. gingivalis* (50 µg/mL: 40 % inhibition; 100 µg/mL: 58 % inhibition). Investigation on *quorum sensing* demonstrated a strong increase of Autoinducer-2 at 100 µg/mL of RA1. Due to the fact that the biofilm mass under RA1 treatment was significantly inhibited, the increased release of AI-2 indicates a counter reaction of the bacteria to the interaction with the extract.

For investigations on potential influence of RA1 on innate host immune response the cytokine release of KB cells after infection with *P. gingivalis* was analyzed. IL-8 levels of KB cells were shown to be slightly decreased by coincubation with RA1 and epicatechin-3-O-gallate-(4β->8)-epicatechin-3-O-gallate (10 µg/mL of RA1: 16 % inhibition; 20 µM of ECG-ECG: 21 % inhibition)

Furthermore, the influence of RA1 on *in vitro* phagocytosis rate of murine RAW 264.7 macrophages and the respective NO production was tested. RA1 significantly stimulated the uptake of zymosan particles (10 µg/mL of RA1: 72 % increase) and the phagocytosis of *P. gingivalis* (0.1 µg/mL: 53 % increase) by RAW 264.7 macrophages. No activation of iNOS by the extract was observed.

Within MTT assay, it was demonstrated that RA1 shows a cytoprotective effect on the viability of the macrophages (0.1 µg/mL of RA1: 24 % increase).

Furthermore, murine RAW 264.7 macrophages revealed a significantly decreased TNF-α release at concentrations of 0.0001 to 1 µg/mL RA1 (1 µg/mL: 9 % inhibition).

For identification of lead compounds and quantification of the active compound epicatechin-3-O-gallate-(4β->8)-epicatechin-3-O-gallate in RA1 and in plant material of *R. acetosa,* a ICH-validated UPLC method was established using epicatechin-3-O-gallate as internal standard. Phytochemical investigations of plant material from one seasonal growth period revealed an enrichment of the active compound epicatechin-3-O-gallate-(4β->8)-epicatechin-3-O-gallate in the fruits of the plant. These results give evidence that the harvesting time of plant material of *R. acetosa* for an extract in the use against *P. gingivalis* is of great importance for the activity of the extract.