Abstract
Within the present study herbal extracts and isolated secondary plant compounds were investigated regarding a potentially antiviral effect towards Influenza A-Virus (IAV). The main focus was on extracts from the herb of *Rumex acetosa* L. (acetone-water, proanthocyanidin-enriched Rumex extract [70 %], DER 15.5:1), from the herb of *Eupatorium perfoliatum* L. (EtOH extract [62 %], DER 10.1:1 and homeopathic mother tincture, according to HAB 2000) as well as from the leaves of *Hedera helix* L. (methanolic Hedera extract [70 %], DER 3.8:1). Furthermore the extracts' antiviral mode of action was investigated and studies characterizing the functionality of effective constituents were conducted.

By means of MTT assay and plaque reduction assay a concentration-dependent inhibition of the IAV replication was shown for all of the four extracts. The IC_{50} values, determined by MTT assay, towards IAV were 2 µg/mL (Rumex extract), 57 and 7 µg/mL (EtOH extract and mother tincture, respectively), and 5 µg/mL (Hedera extract). The CC_{50} values were calculated with 79 µg/mL, 290 and 364 µg/mL, and 480 µg/mL, respectively. Herefrom, selectivity indices (CC_{50}/IC_{50}) of 36 for Rumex extract, 5 and 52 for EtOH extract and mother tincture, respectively, as well as 96 for Hedera extract were calculated.
Rumex extract and mother tincture showed antiviral activity against an Oseltamivir resistant IAV (IC_{50} 37 µg/mL and 73 µg/mL, respectively). In addition, Rumex extract inhibited the propagation of a yellow fever virus vaccine strain.
By means of different modified MTT assays and hemagglutination inhibition test (HIT) as well as in assays with decreased temperature (4°C) to distinguish between viral adsorption and penetration, it was shown that Rumex extract inhibits the viral adsorption to the host cell. Detailed molecular investigations of the polyphenol-rich extract by western blotting, Coomassie® stain and real-time PCR revealed the agglutination of virus particles by the extract via interaction with the viral surface protein hemagglutinin (HA), which is essentially important for the viral adsorption process. At higher concentration levels Rumex extract inhibited also the viral penetration process.
In the context of structure-activity relationships of miscellaneous flavan-3-ols, proanthocyanidins and hydrolysable tannins epicatechin-3-O-gallate-(4β→8)-epicatechin-3-O-gallate was identified as a highly active compound (IC_{50} ca. 15 µM).
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galloylated procyanidin is found as a major constituent of Rumex extract. From structure-activity analysis three structural features were derived, which affect the antiviral activity of proanthocyanidins significantly: a) the amount of pyrogalloyl groups, b) the degree of polymerization and c) mode of linkage of the monomeric subunits. The strong anchorage of the highly active compounds to HA by gallate moieties was confirmed by *in silico* analyses.
In signal transduction assays the NF-κB signaling pathway was not affected by cytotoxic concentrations of Rumex extract (100 µg/mL). In contrast, the Raf/MEK/ERK signaling pathway, which was induced by EGF, was inhibited. However these findings were probably due to unspecific effects that occur only in concentrations by far higher than the antiviral effects.

Also the EtOH extract from *E. perfoliatum* inhibited the viral adsorption to the host cell as was shown by modified MTT assays and HIT. By means of bioassay-guided fractionation and subsequent mass spectrometric analyses six potentially active compounds, thereof presumably three dicaffeoyl quinic acids, were identified in the EtOH extract. The capacity of the anti-IAV active constituents to be precipitable with polyvinylpyrrolidone (PVP) pointed to polyphenolic compounds as active ingredients. The marginal activation of the Raf/MEK/ERK signaling pathway by cytotoxic concentrations of Eupatorium extract (500 µg/mL) is probably due to unspecific effects that occur only in concentrations by far higher than the antiviral effects.
Hedera extract inhibited the viral adsorption to the host cell likewise as was shown by modified MTT assays and HIT. Interference of the extract (250 µg/mL) with the NF-κB signaling pathway was not observed.
By means of bioassay-guided fractionation the antiviral properties of Hedera extract disappeared largely. However, via precipitation of the antiviral active constituents by PVP, these were identified as polyphenolic compounds.