Could the virucidal plant extracts prevent infection of common carp (Cyprinius carpio L.) with CyHV-3 (Cyprinid herpesvirus 3)?

The *Cyprinid herpesvirus 3* (CyHV-3) is a virus which infects common and ornamental carp, koi. The CyHV-3 called also KHV induces a koi herpesvirus disease (KHVD) which results in high mortality reaching up to 100% of carp population. The virus emerged in late 90`s of the XX century and still is an causative agent of a huge economic loss in aquaculture. Scientists are trying to solve this problem for many years. Numerous researches are being done towards stimulation of carp immunity, vaccination and selection of naturally resistant carp individuals for breeding, but still the methods are under investigation and none of them is widely available. The suitable method should be safe for human, carp and for environment, cost-effective and convenient for application in aquaculture.

The pilot study revealed, that ethanolic extracts of St. John`s wort and ribleaf inactivated the extracellular CyHV-3, therefore displayed virucidal activity. These plants are being used in folk medicine for ages and displayed health-promoting properties in human. Experimental application of extracts of St. John`s wort and ribleaf into the feed of atlantic salmon and rainbow trout positively influenced health of those fish. Therefore it can be expected, that the extracts of these plants will be safe for carp. The ethanolic extracts of St. John`s wort and of ribleaf appeared to be very potent *in vitro*, therefore their activity will be explored *in vivo* within this project.

The composition of **plant extracts (PE)** strongly depends on the extraction method and extraction conditions. Furthermore, the differences in composition of PE might result in differences in their biological activity. The St. John`s wort and ribleaf will be extracted at various conditions of ethanolic extraction and Supercritical Carbon Dioxide Extraction ($scCO_2$). The last one method is highly efficient and environmentally friendly, however it is unknown, whether PE obtained by $scCO_2$ extraction will be as potent as PE which were applied in the pilot study, because the pilot study utilised ethanolic PE. The virucidal activity of all PE will be compared *in vitro*. Based on the results of *in vitro* examination, the most suitable extraction method and conditions, providing the most potent virucidal PE will be defined. The composition of all PE will be studied to point-out compounds which could be crucial for the virucidal activity of PE. The activity of those molecules, will be explored *in vitro*, single and in combinations, because of possibility of synergistic activity of compounds of PE. As a result, the information necessary to standardise the virucidal PE will be obtained. The most potent virucidal PE will be applied in experiments *in vivo*.

The major portal of the CyHV-3 entry into carp body is the skin, an organ which is covered with mucus. The mucus delays contact of the virus with the epidermal cells of the skin – thus mucus delays the moment of infection. It seems likely, that when the infected fish spreads virus particles in the environment, those particles could get inactivated by the PE soon enough to prevent the entry of the virus into the epidermal cells of non-infected fish, thus prevent the non-infected fish from infection. To examine this possibility, the PE will be added into tanks with non-infected carp. Subsequently virus-infected fish will be added into these tanks and the spread of infection will be monitored among previously non-infected fish. The activity of PE at various concentrations will be examined to determine the lowest concentration of PE, at which the transmission of the virus from infected to non-infected fish will be disabled. This experiment will provide an indication about maximal possible scale of application of PE for prevention of transmission of the CyHV-3 from infected to non-infected carp (large ponds or smaller tanks used by koi retailers).

The studies in atlantic salmon and rainbow trout revealed immunomodulatory activity of St. Johnswort and ribleaf PE, which have been administered orally in feed. Skin and gills belong to mucosal surfaces of the fish, similarly as guts, therefore it can be expected, that PE present in carp-rearing water might display immunomodulatory activity too. Furthermore, the exposure of mucosal surfaces (skin, gills, guts) to certain antigen of a pathogen might modulate immune response of fish towards this pathogen. Saponins, substances which have been also found in ribleaf, acted as an adjuvant enhancing adaptive immune response in turbot. Based on these findings, it seems likely, that the CyHV-3 released by infected fish and subsequently inactivated by virucidal PE, could act as a mucosal vaccine. Therefore this project will explore the immune response in carp subjected to infection with CyHV-3 in the presence of virucidal PE in the carp-rearing water.

The exploration included in this project will provide basic knowledge, which could be useful for further development of a method for prevention of spreading of the virus in carp population as well as for development of a novel method of vaccination of carp. There exist very high chance, that both these methods will be safe, convenient in application, environmental friendly and cost-effective.