Characteristic of plant extracts inhibit the binding of Shiga toxin to Gb_3 receptors

Diarrheal diseases are caused by many pathogens, including enterotoxin-producing strains of bacteria such as *Escherichia coli, Vibrio cholerae or Shigella spp*. The main virulence factors of the above-mentioned bacteria are the production of AB_5 enterotoxins, such as: cholera (CTX), Shiga (STB), or heat-labile enterotoxins produced by *E.coli* (LTX, LT-II). AB_5 enterotoxins consist of two main domains: the toxic A-subunit and the nontoxic, pentameric B subunits, which recognizes and bind to receptors located in the cell membrane. The STX binds to Gb_3 and Gb_4, while CTX and LTB mainly to GM_1 and $GD_{1.}$ The interaction of gangliosides with the B-subunits of cholera toxin is part of the mechanism in which the A-subunit enters into the cytoplasm of human cells where its toxic action is activated.

Diarrhea has a large economic impact on society and kills around 2.2 million people globally each year with fatalities mainly amongst infants and elderly. In Europe the most frequently bacteria causing diarrhea are different strains of *E.coli*. Some of them can produce Shiga toxin (STEC), what is highly dangerous for human and can cause hemolytic-uremic syndrome. In May 2011 in Germany, *E.coli* infected more than 3,500 people, 855 of whom developed the rare, life-threatening complication hemolytic-uremic syndrome. During this outbreak *E.coli* killed 48 people. The biggest problem during this infection causes antibiotic therapy. When bacteria are treated with antibiotic, they produce a huge amount of Shiga toxin, what can cause HUS and the death. We believe that the knowledge of plants used for centuries for treating diarrhea can help against diarrheal diseases caused by STEC. Numerous studies have validated the traditional use of antidiarrheal medicinal plants. The most frequent assays focus on the antibacterial activity of plants. However, there are published examples showing the interaction and possible inhibition of single plant extracts on CTB or LTX.

During our project we will prepare screening assays that allow **determining a plant extracts inhibit binding Shiga toxin to the receptors**. We hope to find plant extract which will inactivated released Shiga toxins and significantly reduce the risk of HUS and fatal outcomes. We assume that inhibition of binding enterotoxin to receptors will block the toxic action of enterotoxins.

To study antitoxic activity of STX we will use a few methods, which allow us to determine whether plant extract can block binding of Shiga toxin to the Gb_3 receptors on the cell membrane. We will use ELISA, flow cytometry and fluorescent microscope assays. The second aim will be identification of plants active compound, which are responsible for anti-enterotoxin activity of tested by us plants extract. At the end of the project we want to understand the mechanism of interaction between plant active compound and enterotoxins.

The results of the project will deliver numerous scientific benefits. In the end of our project, we will prepare a list of plants extract with anti-Shiga toxin activities, which can improve diarrhea treatment. Also we will find a plants active responsible for antitoxic activity. We hope that obtained results allow understanding the mechanism of interaction between plants and enterotoxins.