***In vitro* cultures of *Trifolium rubens* L. – phytochemical, biotechnological studies and evaluation of biological activity**

For centuries, plants have been used for medicinal purposes. Their valuable and also toxic properties were discovered. It was the beginning herbal medicines (plant medicines, natural medicines, phytopharmaceuticals) were defined by ESCOP (European Society Cooperative of Phytotherapy) as pharmaceuticals used for medicinal purposes, the main components of which are parts of plants (leaves, seeds, flowers, roots, etc.) or vegetable preparations (*e.g.* decoctions, infusions, tinctures). Modern pharmacology is based on them. The rich chemical composition of plants as well as valuable biological properties induce modern chemists to synthesize compounds in the laboratory. Unfortunately, sometimes it is time-consuming, expensive, and the results are not satisfactory. It should also be noted that the therapeutic properties of a given species do not depend on one group of compounds dominating in plant, but on the diversity of their chemical composition. Another problem is the constantly expanding list of rare and endangered species of medicinal plants. That is why it is necessary to carry out phytochemical analyzes, study biological activity and broaden knowledge about alternative methods of obtaining plant material; as biotechnology methods, is so important.

The studies planned under the project are aimed at **better understanding of the *Trifolium rubens* species.** This species is classified as rare or endangered species in several European countries and vulnerable in Poland. *Trifolium rubens* has not been thoroughly tested in terms of the content of active compounds and biological activity. The species of genus *Trifolium* (clovers) have long been used in the traditional medicine of Turkey, Iran, European and American countries.

The valuable therapeutic properties of clovers are determined by its rich chemical composition, in which such compounds as: polyphenol - isoflavones, flavonoids and phenolic acids dominate. These are secondary metabolites with scientifically proven biological activity, including anti-cancer, antioxidant or anti-inflammatory.

The aim of this project is to acquire new and to explore this current knowledge about the chemical composition, biotechnological solutions and biological activity of this plant.

The research will answer the putative **hypothesis about *in vitro* cultures of *Trifolium rubens* as a source of biologically active compounds of therapeutic importance**. The solution to this hypothesis will be an important element in assessing the alternative acquisition of active compounds from the species, which is covered by rare and endangered in European countries.

As part of this project, **the chemical composition of *Trifolium rubens* will be carried out** based on modern chromatographic methods. The chemical composition of **flowers and leaves of plants grown in Poland will be known**.

Importantly, the project will also carry out **research in the field of plant biotechnology**. Plant biotechnology is an important field of biotechnology science, which, from a pharmaceutical point of view, creates remarkable possibilities of using *in vitro* cultures methods in the production of secondary metabolites with therapeutic values. **As part of the project, <u>for the first time</u> the method of *in vitro* cultivation of *Trifolium rubens* will be developed. The chemical composition of biomass extracts of *in vitro* cultures of this species will be known.** Moreover**, <u>for the first time</u> the method of *in vitro* cultivation of *Trifolium rubens* in order to increase the production of pharmacologically important secondary metabolites will also be optimized.**

Based on biological tests, the antioxidant, anti-microbial, anti-inflammatory and anti-cancer properties and inhibition of tyrosinase, collagenase and elastase of plant material extracts and *in vitro* cultures will be assessed.

Comparative evaluation of *in vitro* cultures and parent plant of *Trifolium rubens* will be carried out. The results will explain if the *in vitro* cultures of *Trifolium rubens* can be an alternative to the parent plant.

The obtained results will contribute to the **broadening of knowledge** about the chemical composition and biological activity of *Trifolium rubens* obtained from *in vivo* as well as *in vitro* conditions. The results will answer the question about the possibility of conducting *in vitro* cultures of *Trifolium rubens* as an alternative to the *ex vivo* raw material. Obtained results of biological tests will show whether it is worth using *Trifolium rubens* as a herbal medicinal raw material.