In recent years, interest in plant raw materials has grown in the context of phytotherapy. Consequently, the demand for safe and abundant sources of plant medicinal substances is also increasing. Among the many species that can provide valuable bioactive compounds, knotweeds deserve special attention; these plants are often considered invasive, yet they also possess significant pharmacological potential.

Japanese knotweed (*Reynoutria japonica*) is included in the European Pharmacopoeia as "*Polygoni cuspidati rhizoma et radix*". It has been used for centuries in traditional Asian medicine to treat inflammation, cardiovascular diseases, and infections. Modern analyses have demonstrated that the rhizomes of this plant contain significant amounts of stilbenes, particularly resveratrol and its glycoside, piceid, as well as anthraquinones, which exhibit potent antioxidant and anti-inflammatory properties. Sakhalin knotweed (*Reynoutria sachalinensis*) has been used in traditional Eastern medicine, primarily for treating joint pain and jaundice, however, it has not yet attained pharmacopoeial plant status. The rhizomes of *R. sachalinensis* mainly contain quercetin derivatives and phenylpropanoid esters, including vanicosides A and B, which exhibit anticancer, antiviral, and antidiabetic effects.

The primary goal of the project is to elucidate the mechanisms regulating the synthesis of the most valuable secondary metabolites – stilbenes (resveratrol, piceid) and vanicosides A and B – in two species of knotweed: *R. japonica* and *R. sachalinensis*. Our goal is to pinpoint which parts of the plant are involved in producing these compounds, identify the genes that encode key enzymes, and determine the environmental conditions that optimize the production of these substances. This knowledge will facilitate modifications to cultivation methods or genetic engineering in the future to obtain plant material with an optimized chemical profile.

The project implementation will rely on integrated multiomic analyses – a combination of advanced mass spectrometry (metabolomics) and RNA sequencing (transcriptomics). The initial stage is planned to compare four plant organs (rhizomes, stems, leaves, and flowers). Measurements of the concentrations of resveratrol, piceid, and vanicosides in individual plant organs will be taken, while the activity of genes encoding enzymes from their biosynthetic pathway will be analyzed concurrently. This will allow for an assessment of whether the synthesis and storage locations of these compounds overlap or if they are transported within the plant. The second stage will involve conducting a transcriptome analysis on selected organs of knotweed. By comparing gene expression profiles between the two knotweed species, candidate genes responsible for species-specific production of bioactive substances, including a previously unknown pathway for vanicoside synthesis, will be identified. In the third stage, plants will transition to *in vitro* cultures. Knotweed plants will be grown in liquid media and then subjected to exogenous stress factors, including salicylic acid (SA) and methyl jasmonate (MeJA). These substances mimic natural plant defense signals in response to a pathogen attack. As a result of these experiments, the production of stilbenes and vanicosides, along with the corresponding changes in gene expression, will be monitored. This will help us identify molecular “switches” responsible for promoting or inhibiting the synthesis of the desired metabolites.

The implementation of the described research program will bring numerous benefits. Firstly, the stages of stilbene and vanicoside biosynthesis will be clarified, including the key genes encoding enzymes in these pathways. Secondly, it will provide guidance on increasing the production of valuable substances, for example, by stimulating them with natural elicitors or modifying cultivation conditions. Thirdly, a catalog of genes will be developed for future biotechnological or engineering optimization to obtain plants with an improved pharmacological profile. Ultimately, the project will contribute to the rational use of knotweeds – plants previously considered primarily invasive and harmful – by highlighting their valuable potential as a source of modern herbal medicines.

In the long term, the knowledge obtained may provide a foundation for introducing new phytotherapeutics to the market and for conducting further research on the potential use of invasive knotweed populations to produce valuable bioactive compounds without harming the environment.