METABOLOMIC CHANGES OF IN VITRO RHODIOLA ROSEA SAMPLES AFTER VARIOUS TREATMENTS

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Arctic root (*Rhodiola rosea* L.) is a perennial plant in the family Crassulaceae. The root and the rhizome are used in traditional medicine to alleviate the symptoms of stress and fight fatigue. Many preclinical tests were performed in order to prove the beneficial effects of the plant, which showed promising results, however, further clinical studies are needed to confirm the evidence-based use. On the other hand, the pharmacological tests highlighted that the molecules responsible for the biological effects are phenylalkanoids. Since the species is endangered and cultivation is ineffective, *in vitro* plant production can offer a solution. To increase the yield of the active ingredients, different precursors and elicitors can be applied.

In this study, *R. rosea* plants grown *in vitro* were subjected to six different treatments (cinnamic alcohol, methyl jasmonate, the combination of the two, phenylalanine, yeast extract and iodosalicylate) in order to investigate their effects on metabolic changes. The level of phenylalkanoids were determined by a HPLV-UV method, but to obtain information about the changes of other metabolites too, UPLC-HRMS/MS studies were also carried out. For data dependent analysis, the key metabolites were tentatively identified based on their formula calculated from the exact mass results and their MS/MS spectra. For data independent statistical analysis, all features were applied above an arbitrary threshold level.

Based on the HPLC-UV results, phenylalkanoid levels raised most after cinnamic alcohol, and cinnamic alcohol + methyl jasmonate treatment. During the analysis of the key molecules, more than fifty compounds were identified, namely monoterpenoid glycosides, flavonoids, caffeic acid derivatives, lignans and procyanidins. The group treated with phenylalanine produced significantly more caffeic acid derivatives and galloyl esters. Elicitors methyl-jasmonate and iodosalicylate were absorbed to the *in vitro* plants, but the endogenous metabolites have not changed significantly. Statistical analysis of the data independent results showed that in some cases, intragroup differences had higher impact on the samples, than intergroup treatments.

Based on our investigations, the chemical profile of *in vitro* arctic root can be finetuned. UPLC-HRMS/MS analysis shed light to metabolic changes even in groups, where the level of phenylalkanoids had not increased. Our results also show how *in vitro* plants, as living pharmaceutical factories, can respond differently to even the most similar conditions due to their genetic diversity, highlighting the importance of the quality control of their products.