SCIENTIFIC AND TECHNIC REPORT

Stage 2 - 2015

Elaboration of the functional model for sustainable capitalization of the genetic and phytochemical diversity of the natural populations and experimental cultures of *Arnica montana* L. in northern area of the Romanian Eastern Carpathians

Stage summary

The activities conducted in this phase focuses on interdisciplinary studies regarding the characterization of the natural habitats of *Arnica Montana* L. in the target area (biotic and abiotic components), the bioproductivity assessment in terms of biomass and bioactive phytochemical constituents biosynthesis (on compound groups), the achievement of the experimental cultures, the assessment of the extraction and phytochemical analysis methods. These constitute components of the sustainable capitalization model that will be developed within the project.

The description of the natural habitats of Arnica montana area was achieved for the studied area, represented by the central-nordic area of the Romanian Eastern Carpathians (Stanisoarei, Bistritei, Rarau, Calimani mountains and the Dorna Valley, with altitudes between 700 to 2000 m). For the characterization of the biotic component of the environment, data regarding the vegetation specific to the natural areas studied was collected. Also, in the studied area, the entomofauna characteristic to A. montana specie was identified and characterized (pollinators and pests).

For *the evaluation of the abiotic environmental factors*, the meteo-climatic and pedological factors were studied and *the analysis of the vulnerability maps of A. montana habitats* was achieved. In the next phase these data will be used in the characterization of the optimum niche for the development of *A. montana* species in the studied area, the assessment of the abiotic factors influence on the bioproductivity (biomass) and on the bioactive phytochemical compounds content respectively.

In order to *optimize the extraction and analysis methods* for the bioactive compounds, several extraction parameters and chromatographic methods (HPLC and GC-MS) were tested. For *the assessment of phytochemical diversity*, for the samples harvested from the studied areas, analysis were performed differentiated by plant organ type (flowerheads, leaves, roots and rhizomes), several classes of bioactive compounds being envisaged – sesquiterpen-lactones, phenolic compounds (phenolic acids and flavonoids) and volatile oil.

The phytochemical analysis for phenolic acids and flavonoids content had as purpose the evaluation of the bioproductive potential for these bioactive compounds, for *A. motana* plans harvested from the studied are, for the assessing the holistic capitalization of the plant biomass for the domestication of the species. Experiments were developed to test the *optimum cultivation conditions by using unconventional methods (in vitro regenerated plants) and by initiating the conventional cultures*.

In order to develop the methodology for the quantitative determination of several metabolites in A. montana extracts by NMR were developed studies on: extraction and fractionation of the plant material harvested from natural populations; test for the assessment of some metabolites by NMR; the assessment of the discriminatory possibilities of the samples on the base of NMR spectroscopy.

Also, the assessment of the phytochemical structure by HPTLC, for the samples harvested from the natural populations (qualitative analysis/ compound identification), preliminary studies for obtaining plant extracts enriched in phenolic acids, flavonoids and phytosterols respectively was achieved. The evaluation of the extractive solutions achieved by Pharmacopoeia methods, through conventional extraction methods, with selective solvents for the compounds classes of interest provided preliminary information on the solvents and extraction parameters to be used in advanced experiments for obtaining plant extracts enriched in phenolic acids, flavonoids and phytosterols.