Dandelion (*Taraxacum officinale* (L.) Weber ex F.H.Wigg.) is a herbaceous perennial widespread in temperate climates, usually growing on pastures and wastelands. For centuries, this plant has been used in folk medicine in many countries as an adjuvant in the treatment of various ailments, such as liver disease, digestive problems, diabetes, skin inflammation, arthritis and rheumatic diseases. In addition to being used as pharmaceuticals, all parts of the plant (roots, leaves, flowers) can be processed into various food products - salads, infusions, coffee substitutes, syrups, cakes, tinctures and wines. Currently, dandelion root and herb are classified as a Pharmacopoeial and GRAS (Generally Recognized As Safe) material and are approved for pharmaceutical use in the form of ethanol extracts and infusions by the European Medicines Agency and the Federal Drug Administration.

Over the past 15 years, dandelion root (*Taraxaci radix*, TR) has been the subject of several scientific studies investigating its inhibitory effect on cancer cells; several different research groups have worked on this topic and the results have been published in prestigious scientific journals. The action of *Taraxaci radix* extracts (mainly aqueous and ethanolic) has been tested on several different types of cancer - breast (MCF7), colon (HCT116, HT29), gastric (BGC823, SGC7901), leukemia (CMML), liver (HepG2), melanoma (A375, G361) and prostate (LNCaP), obtaining pronounced cytotoxic effects as well as specificity for some cell lines. Despite many reports that investigated the broad antitumor activity of dandelion root extracts, the active ingredients responsible for the observed inhibition and the detailed mechanisms underlying it remain poorly described/unknown so far. Only one publication (Ovadje et al. 2016) has made efforts to identify the tumor-suppressing active ingredients of TR out of all available. The authors attributed this effect to the pentacyclic triterpenoid fraction, but interestingly, the tested single components of the fraction (α- and β-amyrin and lupeol) showed moderate/no activity. Moreover, given the rather low levels of triterpenoids in the dandelion root (based on literature data and own observations) and their chemical nature - present mainly as aglycones devoid of sugar chains (which makes extraction difficult/impossible with water), the uncertainty regarding the identification of the bioactive component of TR becomes even greater. Phytochemically, dandelion root is a complex mixture of many different natural products; the main biologically active components of TR are hydroxycinnamic acids, sesquiterpene lactones (SLs), pentacyclic triterpenoids, inositol phenylacetate esters (PIE) and polysaccharides. Nevertheless, new papers describing the presence of previously unknown metabolites in this plant material are systematically published, as exemplified by the publication of our research team on the tentative identification of SLs-proline conjugates (Jedrejek et al. 2019). In addition, there are large gaps in knowledge of the quantitative content of bioactive metabolites in the dandelion root, even for polyphenolic compounds such data is limited and for other natural TR products (SLs, pentacyclic triterpenoids and PIE) are simply not available.

Considering the promising cytotoxic activity of dandelion root extracts in many of the described studies and the incompleteness of knowledge about the active compounds responsible for these effects, the main goal of the project is to undertake efforts to distinguish and identify actual TR metabolites with tumor-inhibitory activity. The project will use ecological plant material collected in the Kazimierz Landscape Park. *Taraxacum* extracts and fractions will be prepared in an environmentally friendly manner, using 'green solvents' (mainly ethanol) and ultrasound-assisted extraction technique. About 10 TR preparations, varying in composition, will be tested in phase I of the project for anti-proliferative properties using several tumor cell lines, including breast, lung and ovarian cancer, as well as normal human keratinocytes. To test tumor inhibition, a luminescence method (CytoTox-Glo™ assay) will be used, in which a luminogenic peptide substrate is applied to cells from the control and test groups to measure protease activity. The use of metabolomics, based on LC-HR-QTOF-MS data and multivariate statistical analyzes (MetaboAnalyst platform), will highlight the presence of chemical markers (metabolites) in the set of compared samples. Then, a classical methodology of bioassay-guided isolation will be used to unquestionably identify TR biomarkers, as well as to verify whether both strategies will ultimately indicate the same active compounds. The own database of TR metabolites, spectroscopic techniques and open databases of natural compounds will be used to identify chemical markers of active preparations. Knowledge gaps in the quantitative content of bioactive metabolites in dandelion root and the variability of plant material in the annual, seasonal and population cycle will be filled using targeted and non-targeted metabolomics, based on LC-PDA-MS/MS data and multivariate exploratory and discriminant analysis.