

*Monotropa hypopitys* L. (MH, pinesap, family Ericaceae) is an unusual perennial without photosynthetic processes that obtains all its vital substances from parasitic mycorrhizal fungi and indirectly from their autotrophic partners. The genus *Monotropa* has only two representatives (MH and *M. uniflora*), both of which resemble fungi more in lifestyle and appearance (visible only during flowering; leafless flowers similar to fungal fruiting bodies) than other vascular plants. *Monotropa* spp. have a relatively wide distribution (forests of almost the entire northern hemisphere), unlike many other rare/endemic mycotrophs, which may indicate their lower habitat requirements. However, *Monotropa* plants are not common species, i.e. they occur in large numbers but only in limited locations. There are few studies on ecological and trophic interactions of MH, and close mycotrophic relationships have only been described with two *Tricholoma* species, which are known mycorrhizal companions of autotrophic *Pinus* and *Salix*. Nothing is known about the influence of other habitat characteristics, such as soil chemical properties, or the existence of trophic interactions with other trees (e.g., oak) and/or other known mycorrhizal partners of pine and willow. Cultivation methods for *Monotropa* spp. have not yet been developed, so they can only be found in the wild. In addition, it has been observed that mycotrophs are able to enter short or long (even several years) dormant periods, the reasons for which have not been further elucidated. The above factors make it very difficult for researchers to monitor and study these plants. According to several reports, *Monotropa* plants were once used in folk/tribal medicine as sedatives, hypnotics, anticonvulsants and epileptics, analgesics and anti-inflammatories, and to promote wound healing. However, to date, there is no biomedical or ethnobotanical research or published literature confirming the above biological effects and the use of *Monotropa* for any health-related purpose. The state of knowledge on the phytochemistry (including toxicology) of *Monotropa* spp. is similarly poor, as there are no studies or publications in this area. Metabolomic studies of MH are important for a deeper understanding of ecological relationships (the aspect of metabolite sequestration) and to determine the potential for use in phytopharmaceuticals.

The project is described in 10 tasks, which can be divided into three main research activities: A) ecological determinants of MH and its environmental relationships (analysis of different botanical, chemical and microbiological characteristics); B) metabolomic profiling and chemometric analyses of plant materials and fungal isolates from different sites; C) *in vitro* bioactivity of MH metabolites in relation to reports of use in folk medicine. The study will use samples collected from 3 different natural MH sites (plants and soil, habitat A) and 2 negative sites (soil, habitat B) for two consecutive seasons (2024-25). Key habitat attributes, including photos and botanical and mycological characteristics, will be monitored for several months each year. The mycobiome of soil samples will be subjected to molecular studies and individual mycelia will be isolated, further identified by genetic analysis (DNA sequencing) and characterized microscopically and metabolically (Biolog® system and LC-MS profiling of metabolites). In addition, the soils will be analyzed for physicochemical parameters, including pH, humus, macro- and micronutrients, and other natural products (e.g., amino acids, phenolics). The above results will be the starting matrix for chemometric analyses (MH vs. negative site) to extract and identify distinctive environmental features, incl. positively/negatively correlated with MH habitat. LC-HR-MS analyses and targeted isolation of unidentified relevant metabolites will allow detailed phytochemical profiling of different MH collections and populations in southeastern Poland. Studies of MH phytochemicals are important to determine the potential of *Monotropa* for use in phytopharmaceuticals, as well as to elucidate the ecological contexts of mycotrophs. A metabolomic approach will be used to confirm the likely simplification of secondary metabolism and sequestration of host-specific metabolites, as observed in other parasitic plants. Several MH formulations with specific phytochemicals and individual metabolites will be developed using chromatographic techniques and then tested for various biological properties. Among the tests will be panels devoted to cytotoxic (blood cells), anti-inflammatory (COX, LOX, cytokines, urokinase receptor), coagulation and fibrinolysis effects, and inhibition of enzymes active in pathophysiological states (AChE, NO synthase, G6PD). The phytochemical and biochemical results will provide the starting matrix for chemometric analyses aimed at identifying pinesap metabolites that exhibit distinct and directional health-promoting effects.

The project is eminently cognitive in nature and is characterized by interdisciplinarity due to its multifaceted approach and various defined research hypotheses. Given the scarcity of evidence and remaining scientific literature, its overall goal is to fill research gaps regarding the ecological, trophic, and phytochemical aspects of the life of Monotrophoidae mycotrophs, as well as to lay the groundwork for their use in phytopharmaceuticals.