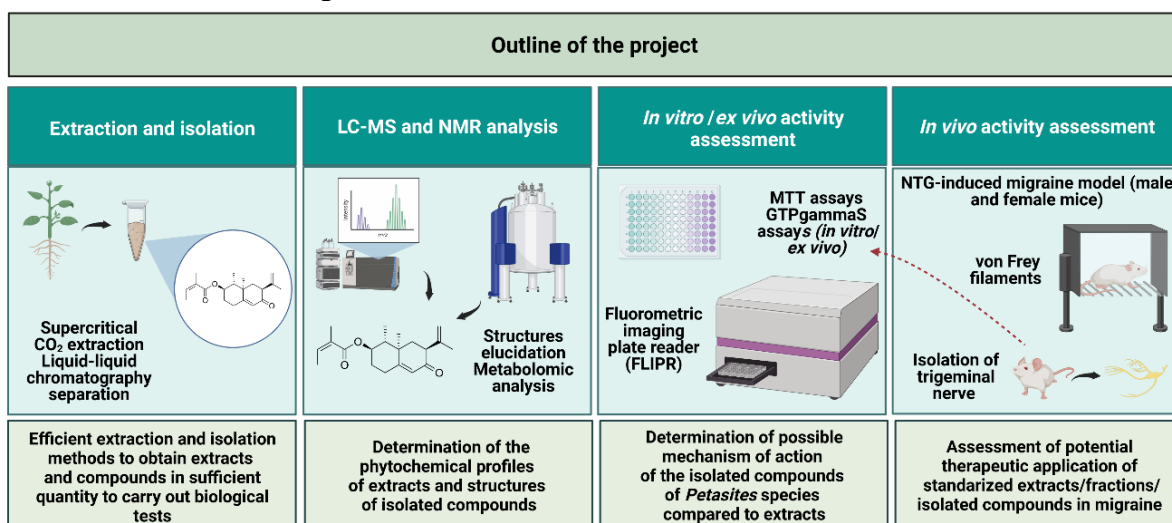


Evaluation of butterbur (*Petasites* spp) for the antimigraine potential: Isolation of bioactive secondary metabolites (petasins) and pharmacological characterization

Plants of genus *Petasites* (butterbur, *Asteraceae*) have been known in the traditional medicine since ancient times in treatment of ailments such as migraine, asthma, allergic rhinitis, or hypertension. Numerous clinical trials confirm activity of *P. hybridus* extracts in central nervous system diseases, mainly anti-migraine effect. Other species known in ethnopharmacology are *P. japonicus*, *P. tricholobus*, and *P. formosanus*. However, there are many unknown species (e.g. *P. kablikianus*), which are awaiting investigation and they can be new source of unknown active compounds.

Petasins (eremophilane-type sesquiterpenes) are considered as the main bioactive components of *Petasites* extracts. Whereas, the activity and mechanism of action of individual compounds is not fully understood, and compounds are not available for the commercial purposes. Therefore, developing efficient methods for the isolation of biologically active compounds from *Petasites* is crucial. Moreover, crude extracts of widely used butterbur contain hepatotoxic and carcinogenic pyrrolizidine alkaloids (PAs).

Despite continuous research on the bioactive principles in *P. hybridus* extracts, there is a need for the isolation and characterization of all bioactive principles. Thus, the aim of this project is to elaborate separation methods for the isolation of the active metabolites from *P. hybridus* as well as to evaluate their behavioral effects and anti-migraine mechanisms of action.



This interdisciplinary project consists of studies, which will provide information about efficient extraction, isolation and identification of the compounds using modern chromatographic methods, focusing on **supercritical CO₂ (scCO₂) extraction and liquid-liquid chromatography (LLC)**. It will be for the first time when constituents of *Petasites* will be isolated using LLC. The project combines phytochemical methods with *in vitro* and *in vivo* experiments. The use of efficient methods for the extraction and pure compounds eliminates the risk of contamination with PAs. Additionally, *Petasites* species will be analyzed by **liquid chromatography hyphenated with high-resolution tandem mass spectrometry (LC-HRMS/MS)** and **1D and 2D nuclear magnetic resonance (NMR)** techniques in search of other sources of active compounds and to elaborate phytochemical profiles of unknown *Petasites* species.

In the current project we will study the mode of **anti-migraine** action focusing on **ion channels** and on the modulation of the **CGRP pathway**, which seems to be involved into the mechanism of anti-migraine activity of butterbur.

The standardized extracts/fraction and/or isolated compounds will be tested *in vivo* in mice using a **nitroglycerin (NTG)-induced migraine model**. **Both male and female will be tested as sex differences in migraine prevalence have been known but not yet explained.**

This kind of research has not yet been developed for tested compounds. **In the frame of the project an efficient isolation methods of petasin derivatives will be elaborated and antimigraine activity together with proposed mechanism of action will be assessed.**