

## **Gut-brain-axis perspective on the impact of Cannabis flos on the blood-brain-barrier**

Inflammation within the Central Nervous System (CNS), known as **neuroinflammation**, is considered a key factor in the pathogenesis of neurodegenerative diseases (such as Parkinson's and Alzheimer's diseases) and mood disorders, including depression, anxiety, and cognitive dysfunctions. The development of inflammation in the CNS has multifactorial etiology, but the most commonly mentioned factors include a decline in the integrity of the blood-brain barrier and inflammation initiated by non-neuronal microglial cells. In recent years, increasing attention has been given to the bidirectional communication pathway between the gut microbiota and the CNS, referred to as **the microbiota-gut-brain axis**. The gut microbiota is an integral part of the organism that enables its proper functioning. It has been shown that maintaining a healthy gut microbiota is a crucial factor in regulating CNS homeostasis. Concurrently, observed changes in the composition of the gut microbiota during neurodegenerative diseases and mood disorders highlight the need for new potential therapeutic implications that consider the bidirectional nature of this communication.

The diversity of the gut microbiota largely depends on many factors, not only dietary components and synthetic drugs but also natural products and specialised plant metabolites used for their medicinal properties. On the other hand, in recent years, more attention has been paid to the role of the gut microbiota in assessing the efficacy and mechanisms of action of established therapies, including those based on plant-derived products. An essential aspect of research on the pharmacokinetics and bioavailability of orally administered herbal medicines is the formation of **postbiotic metabolites**, often with greater therapeutic potential compared to the parent compounds. From this perspective, including the gut microbiota as a critical factor influencing the medicinal properties of herbal remedies offers an opportunity to fill the knowledge gap resulting from inconsistencies between the pharmacokinetics of individual specialised plant metabolites and the effects observed in *in vivo* studies.

*Cannabis sativa* L. is a plant species that has been studied for many years for its potential use in the treatment of various medical conditions, such as chronic pain, vomiting connected with chemotherapy, epilepsy, and spasticity associated with multiple sclerosis. The pharmaceutical raw material used in medicine and therapy consists of dried female inflorescences without leaves and stems, which are a source of over 450 compounds, including cannabinoids, flavonoids, and terpenes, with potential anti-inflammatory, antioxidant, and analgesic properties. **Due to the complex chemical composition and properties of its specialised metabolites (high lipophilic potential), the use of Cannabis flos in the treatment of neuroinflammatory-based conditions, including neurodegenerative diseases and mood disorders, has gained significant attention in recent years.**

The most common route of *Cannabis flos* administration is inhalation, practised in the form of vaporisation. Significant exclusions limiting the use of this plant material by this route are respiratory diseases (asthma, chronic obstructive pulmonary disease, chronic cough), as well as trouble handling vaporiser devices, non-compliance, or patients' personal preferences. Consequently, Polish Pharmaceutical Law includes the use of oral forms of *Cannabis flos* administration, such as dried inflorescences intended for pharmaceutical preparations, as well as infusions, pharmaceutical tinctures or cannabis resin.

**At the same time, the established role of the gut microbiota in modifying the activity and bioavailability of oral forms of phytotherapeutics suggests potential interactions between oral forms of Cannabis flos administration and the gut microbiota. Unfortunately, studies on the bidirectional interaction between Cannabis pharmaceutical formulations and human gut microbiota, though necessary for understanding their mechanisms of action, are significantly limited.**

The planned research aims to provide scientific evidence evaluating the metabolism of available therapeutic oral preparations of *Cannabis flos* (infusions, tinctures), as well as micronised/non-micronized raw materials with human gut microbiota. The obtained results will allow for the identification of forms of raw material administration that initiate the formation of the greatest diversity of postbiotic metabolites with potential pharmacological effects, as well as those most favourably affecting the diversity of human gut microbiota. The aim of the planned studies is also to answer the question of whether and how *Cannabis flos* postbiotic metabolites impact the functions of the blood-brain barrier and the inflammatory phenotype of non-neuronal microglial cells. The obtained results will indicate the potential mechanism of action of oral forms of cannabis administration in the context of their impact on neuroinflammatory conditions. The implementation of the proposed project may also serve as a first step in developing new therapeutic solutions based on postbiotic metabolites of *Cannabis flos*.