ABSTRACT

Hemp (Cannabis sativa L.) has been already cultivated by prehistoric societies due to its diverse usability. Today, it still attracts significant attention primarily for its medicinal effects and psychoactive properties. The latter have led to the long-standing stigmatization of hemp, with its use often strictly limited or even prohibited. However, in recent years, the medicinal effects of hemp are once again coming to attention, and restrictions related to its cultivation and use are largely loosening. The unique medicinal properties of hemp, as well as its psychoactive effects, are attributed to the presence of cannabinoids, secondary metabolites characteristic (almost exclusively) to hemp. Hemp also contains other groups of secondary metabolites, mainly terpenes, and various phenolic compounds. These two groups of compounds have proven bioactive effects, and there is also known synergistic action between terpenes and cannabinoids (»entourage effect«).

The doctoral thesis primarily focuses on the analytical aspects of hemp study, furthermore it also addresses the theme of increasing the water solubility of key cannabinoids, which is important in terms of enhancing their bioavailability. The research work can be divided into four main sections.

In the first part, a gas chromatography method for the simultaneous determination of cannabinoids and terpenes was developed. Event though numerous reliable chromatographic methods for determining both groups of compounds in hemp already exist, our approach is innovative mainly because both groups of metabolites can be determined simultaneously. The entire process, from sample preparation to chromatographic analysis, is fast and simple, but also robust and accurate enough to have broad practical usability.

The second part addressed isolation of acidic forms of cannabinoids from the plant. Acidic forms are generally quite unstable and commercially less accessible. However, they posses proven medicinal properties, making them interesting for further studies and applications. During the research work, isolation of cannabidiolic acid was optimized. For the purposes of our research, the synthesis of cannabidiolic and cannabigerolic acids from cannabidiol and cannabigerol proved to be a practical and simpler alternative, compared to isolation.

The third part of the doctoral thesis focused on studies with the purpose of increasing the water solubility of cannabinoids, which are inherently poorly soluble (or almost insoluble) in water. An approach involving the formation of a complex with glucosamine was developed, significantly increasing the water solubility of both acidic and decarboxylated forms of cannabinoids. The formation of the complex was demonstrated using multiple complementary analytical techniques, and the systematic increase in the water solubility of various cannabinoids, both isolated and present in the extract, was evaluated. It was found that the formation of the complex with glucosamine also stabilizes the acidic forms of cannabinoids.

The final, fourth part of the doctoral thesis was focused on phenolic compounds. While these are the most prevalent group of secondary metabolites in nature, and they are present in hemp as well, hemp phenolics have been relatively understudied due to the general focus on cannabinoid

and terpene research. Within the research, thin-layer and liquid chromatography methods were developed for the determination of phenolic compounds, and identification was performed using mass spectrometry. Water residues from hemp steam distillation, commonly used to obtain essential oil, were also evaluated as potential sources of phenolic compounds.

Keywords: hemp (*Cannabis sativa* L.), cannabinoids, terpenes, phenolic compounds, gas chromatography (GC), high performance liquid chromatography (HPLC), mass spectrometry, isolation of cannabinoids, cannabinoid water solubility