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Abstract

Determination of CBD in Ethanol Extracts Prepared from Hemp (*Cannabis sativa* L.) Cultivar Beniko Using Dynamic Maceration and Ultrasound Assisted Extraction †

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Abstract: Hemp (*Cannabis sativa* L.) has been known as a source of fibres, oil and medicines since ancient times (4000 B.C.). Currently, there is an increasing interest in fibrous hemp as a source of compounds with pharmacological activity. Cannabis is rich in biologically active compounds such as terpenes, flavonoids, sugars, alkaloids and the most known unique cannabinoids including psychoactive Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THCA) and non-psychoactive cannabidiol (CBD), cannabidiolic acid (CBDA). Fast and increasing interest in using low-THC (<0.2%) hemp requires the development of efficient methods for extracting bioactive substances. Conventional methods of cannabinoid extraction, such as Soxhlet extraction or maceration, require longer extraction times and large amounts of solvent. The addition of ultrasounds or mixing may support the process of extracting bioactive compounds. The aim of the study was comparison of CBD extraction methods from *Cannabis sativa*. For extraction with ethanol (40%, 60% and 80%) were selected fresh panicles of monoecious hemp variety Beniko (2.55% CBD and 0.06% THC) allowed to cultivation in Europe. After harvesting, the panicles were frozen in liquid nitrogen and stored in a freezer (−20 °C). For extraction, dynamic maceration was used during which the plant material was shaken in the dark (100 rpm, 72 h) and ultrasound assisted extraction (45 Hz, 0.5 h). Analysis showed the highest content of CBD (361.3 μg/mL) during extraction with 80% ethanol combined with ultrasound. On the other hand, dynamic extraction with 60% ethanol allowed to obtain 352.7 μg/mL of CBD. In the case of ultrasound assisted extraction was observed significantly lower content of CBD at the level 303.4 μg/mL for extraction with 40% and 301.4 μg/mL for 60% ethanol in comparison with the higher content of CBD (361.3 μg/mL) obtained during extraction with 80% ethanol. Differently, dynamic maceration with 40% ethanol showed significantly low content of CBD on the level 252.6 μg/mL and application of 60% ethanol increased CBD content to 352.7 μg/mL which was significantly high, whereas extraction with 80% ethanol showed 331.9 μg/mL of CBD. In addition extraction with dynamic maceration revealed significant differences in level of THC, and in 40% ethanol was observed the lowest content THC (10.0 μg/mL) and in 80% ethanol the highest content of THC (17.7 μg/mL). Ultrasound assisted extraction showed not important changes in the level of THC from 11.4 μg/mL in 40% ethanol to 15.6 μg/mL in 80% ethanol respectively. The use of ultrasound supports the extraction by faster mass transfer and uniform mixing, which accelerates the extraction of compounds, what was showed at the extraction with 80% ethanol. A similar effect at a lower ethanol concentration (60%) was observed when dynamic maceration was used. The obtained results indicate that the most efficient method of extraction is ultrasound assisted extraction, however, dynamic maceration with shaking may be an alternative method.

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These methods can be used to obtain high-quality extracts containing bioactive compounds for pharmaceutical purposes and food production.

Keywords: *Cannabis sativa*; fibre hemp; ethanol extract; CBD; THC

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