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SUSTAINABLE POSTHARVEST AND
FOOD TECHNOLOGIES**

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ENCAPSULATION OF CANNABIDIOL-RICH HEMP EXTRACT

Ana ŽUGIĆ¹, Vanja TADIĆ¹, Marija TASIĆ KOSTOV², Ivana NEŠIĆ²

¹*Department of Pharmaceutical Research and Development, Institute for Medicinal Plant Research
“Dr. Josif Pančić”, 1 Tadeuša Koščuška Street, Belgrade Serbia*

²*University of Nis, Faculty of Medicine, 81 Zorana Djindjica Boulevard, Nis, Serbia*

Contact: azugic@mocbilja.rs

Hemp is grown as an agricultural crop, characterized by low content of psychoactive Δ -9 tetrahydrocannabinol (THC, less than 0.3%). Numerous research caused the increase in scientific knowledge regarding hemp composition and health benefits, whereby much attention has been focused on non-psychoactive cannabinoids, especially cannabidiol (CBD) that exert potent anti-inflammatory, and neuro-protective effects. These findings induced an intense growth in utilization of CBD-based nutritional supplements and cosmetics. Recently, supercritical extraction with carbon dioxide (SCO₂) was shown to extract notable amounts of CBD from hemp compared to the conventional extractions. However, CBD possesses demanding physicochemical characteristics, such as sensitivity against light, rapid degradation and poor water solubility. These features make CBD a suitable candidate for encapsulation into advanced delivery systems (DSs).

In the current investigation, SCO₂ extraction was used for the production of CBD-rich hemp extract using inflorescence of *Cannabis sativa* L., Cannabaceae (*var.* Helena). Comprehensive chemical profiling of the obtained hemp extract was achieved by HPLC analysis. Afterwards, hemp extract was encapsulated into nano-sized DSs that were characterized by determination of mean diameter, polydispersity index (PDI) and zeta potential (assessed by photon correlation spectroscopy, PCS), encapsulation efficiency, as well as pH and conductivity of the obtained dispersion of nano-DSs. To test the preliminary physicochemical stability of the obtained nano-DSs, PSC measurements, as well as pH and conductivity measurements were repeated after 1 month of room temperature storage.

CBD content was 13.02 % in the obtained hemp extract. Encapsulation efficiency of CBD in the nano-DSs was quite high and amounted 99.99%. Their size was 243.30±1.77 nm, with low PDI of 0.213±0.016 indicating uniform particle distribution, while zeta-potential was 65.53±2.65 suggesting good kinetic stability. pH value was 4.62, while conductivity was 423 μ s/cm. Repeated measurements of size, PDI, zeta potential, pH and conductivity did not change significantly compared to the initial measurements, indicating satisfactory preliminary physico-chemical stability of the developed nano encapsulants of CBD-rich hemp extract.

In conclusion, hemp extract rich in CBD was successfully encapsulated into nano-sized DSs which were proven to enable satisfactory preliminary physico-chemical stability of CBD, otherwise sensitive to environmental conditions. Further investigations of biological activities of the developed nano-vehicles are needed to enable their potential application in the food, cosmetic and pharmaceutical industry.

Key words: *supercritical carbon dioxide extraction, nano-sized delivery systems, preliminary physico-chemical stability.*

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INKAPSULACIJA EKSTRAKTA KONOPLJE OBOGAĆENOG KANABIDILOM

Ana ŽUGIĆ¹, Vanja TADIĆ¹, Marija TASIĆ KOSTOV², Ivana NEŠIĆ²

¹Odsek za farmaceutska istraživanja i razvoj, Institut za proučavanje lekovitog bilja "Dr Josif Pančić", Tadeuša Košćuška 1, Beograd, Srbija

²Univerzitet u Nišu, Medicinski fakultet, Bulevar Zorana Djindjica 81, Niš, Srbija

Contact: azugic@mocbilja.rs

Konoplja se gaji kao poljoprivredna kultura sa niskim sadržajem psihoaktivnog jedinjenja Δ -9 tetrahidrokanabinola (THC, manje od 0,3%). Dosadašnja brojna istraživanja konoplje dovela su do naučnih saznanja u vezi sa njenim sastavom i zdravstvenim prednostima, pri čemu se sve više pažnje usmerava na nepsihoaktivne kanabinoide, posebno kanabidiol (CBD) koji ispoljava snažno antiinflamatorno i neuroprotektivno delovanje. Navedena naučna otkrića dovela su do intenzivnog porasta upotrebe dodataka ishrani i kozmetike na bazi CBD-a. Nedavno je pokazano da natkritična ekstrakcija ugljenik(IV)-oksidom (NKE) može da izdvoji značajnu količinu CBD-a iz konoplje u poređenju sa konvencionalnim ekstrahiranjima. Međutim, CBD poseduje nepovoljne fizičko-hemijske karakteristike, kao što su osetljivost na svetlost, brza degradacija i loša rastvorljivost u vodi. Ove karakteristike čine CBD pogodnim kandidatom za inkapsulaciju u napredne sisteme isporuke (SI).

U ovom istraživanju, NKE iz cvasti *Cannabis sativa* L., Cannabaceae (var. Helena) je korišćena za proizvodnju ekstrakta konoplje bogatog CBD-om. Sveobuhvatno hemijsko profilisanje dobijenog ekstrakta konoplje postignuto je HPLC analizom. Nakon toga, ekstrakt konoplje je inkapsuliran u SI nano veličine, koje su okarakterisane određivanjem srednjeg prečnika, indeksa polidisperznosti (PDI) i zeta potencijala (procenjenog foton korelacionom spektroskopijom, FKS), određivanjem efikasnosti enkapsulacije, kao i pH i provodljivosti dobijene disperzije nano-SI. Da bi se ispitala preliminarna fizičko-hemijska stabilnost FKS merenja, kao i merenja pH i provodljivosti su ponovljena nakon 1 meseca čuvanja uzoraka na sobnoj temperaturi.

U dobijenom ekstraktu konoplje sadržaj CBD-a bio je 13,02 %. Efikasnost inkapsulacije CBD-a u nano-SI je bila prilično visoka i iznosila je 99,99%. Njihova veličina je bila $243,30 \pm 1,77$ nm, sa niskim PDI od $0,213 \pm 0,016$ što je ukazalo na ujednačenu distribuciju čestica, dok je zeta-potencijal bio $-65,53 \pm 2,65$ ističući dobru kinetičku stabilnost. pH vrednost je bila 4,62, dok je provodljivost bila $423 \mu\text{S}/\text{cm}$. Rezultati ponovljenih merenja veličine čestica, PDI, zeta potencijala, pH i provodljivosti nisu se značajno razlikovala u poređenju sa rezultatima početnih merenja, što je ukazalo na zadovoljavajuću preliminarnu fizičko-hemijsku stabilnost razvijenih nanoinkapsulanata ekstrakta konoplje bogatog CBD-om.

U zaključku, ekstrakt konoplje bogat CBD-om uspešno je inkapsuliran u SI nano veličine za koje je pokazano da obezbeđuju zadovoljavajuću preliminarnu fizičko-hemijsku stabilnost CBD-a, inače osetljivog na ambijentalne uslove. Potrebna su dalja istraživanja bioloških aktivnosti razvijenih nano-SI kako bi se omogućila njihova potencijalna primena u prehrambenoj, kozmetičkoj i farmaceutskoj industriji.

Ključne reči: natkritična ekstrakcija ugljen-dioksidom, sistemi za isporuku nano veličine, preliminarna fizičko-hemijska stabilnost.

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