PHARMACEUTICS

ENCAPSULATION EFFICIENCY OF ROSA CANINA L. EXTRACT WITH γ- AND 2HPβ-CYCLODEXTRINS

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Abstract

The research found that adding cyclodextrins to *Rosa canina* (Regel) Briq. (RC) extract made it more effective as an antioxidant. The results showed that encapsulation significantly improved the antioxidant activity of *Rosa canina* extract. We can attribute this improvement to the increased solubility and protection of bioactive compounds in cyclodextrin complexes. The results of this study highlight the potential use of cyclodextrin capsules as a strategy to increase the antioxidant activity of natural extracts.

Keywords: bioavailability, antioxidant, cyclodextrins, encapsulated formulations

Introduction

Extracts from medicinal plants differ from drugs in terms of their naturalness, absence of additional chemical components, and pure purity. The presence of therapeutically important biologically active substances in medicinal plant extracts leads to their widespread use in medicine and pharmaceutical industries. The term "main active substances" refers to the biologically active chemical compounds of the plant that have therapeutic value. These substances are frequently found in the form of alkaloids, glycosides, coumarins, essential oils, flavonoids, vitamins, lignans, additives, and other substances characteristic of certain plants; they are used as sedatives, pain relievers, blood pressure reducers, in disorders of various metabolic processes, and in cardiovascular diseases. These substances have a positive effect on various conditions within the body. However, these bioactive compounds are often a challenge because they can have low solubility and stability in the gastrointestinal tract. To address this issue, researchers have explored various methods to improve the bioavailability of plant extracts, including encapsulation with cyclodextrins **[1]**. Cyclodextrins are cyclic oligosaccharides composed of glucose units that form a hydrophobic cavity. This cavity can accommodate hydrophobic compounds, such as the bioactive compounds found in plant extracts **[2]**. By encapsulating plant extracts with cyclodextrins, their antioxidant activity can be enhanced and their bioavailability improved **[3]**. This encapsulation helps to protect the bioactive compounds from degradation and improves their solubility in aqueous environments, making them more readily absorbed.

Today, the world is witnessing the creation of new drugs, the improvement of existing ones, and the delivery of active substances to the human body, which increases their bioavailability and reduces the dosage. Using cyclodextrins and their derivatives to protect bioactive compounds from the environment can make the products work better and protect the compounds from different conditions.

The main purpose of this study was to obtain cyclodextrin encapsulated forms of *Rosa canina* (Regel) Briq. (RC) dry extract and to learn the effect of cyclodextrin type and encapsulation method on antioxidant efficiency and total flavonoid content.

Materials and methods

Rosa canina (Regel) Briq. (RC) dry extract was from Rosa canina (Regel) Briq. hips (locality Tashkent region, Uzbekistan, harvested August-November, 2023) provided by Ltd. BIOTON (Tashkent, Uzbekistan). Gamma-cyclodextrin (γCD) and 2-hydroxypropyl-beta-cyclodextrin (HPβCD) were purchased from CYCLOLAB (Hungary). RC/CD encapsulated forms were obtained by co-evaporation, freeze-drying methods and physical mixture was prepared as well. *Co-precipitation method:* RC, γCD and HPβCD were carefully weight in proportion RC/CD 1:1 (w:w), dissolved separately in equal volume of distilled water, then liquids were mixed and kept at rotary shaker for 12 hours (50±2°C). The samples then was dried at 20±2°C. *Freeze-drying method:* RC, γCD and HPβCD were carefully weight in proportion RC/CD 1:1 (w:w), dissolved separately in equal volume of distilled water, then liquids were mixed and kept at freezer at -18°C until frozen. Samples then were subjected to lyophilization in a freeze-drier (FD-18, Meditry Instrument Co.Ltd, China) for 24 h to obtain a powder. *Physical mixture:* RC/CD were mixed in proportion RC/CD 1:1 (w:w) and vigorously stirred in an agate mortar up to 5 mins.

Total flavonoid content (TFC) was analyzed according to [4]. Quercetin was used for calibration curve.

Total antioxidant capacity (TAC) was determined by phosphomolybdenum assay (PA) [5]. TAC was expressed as mg/g DW ascorbic acid (AA) equivalent.

Chelating capacity was determined by ferric reducing antioxidant power (FRAP) according to Chaves et al., 2020 [6]. FRAP results were expressed as mg/g DW ascorbic acid (AA) equivalent.

DPPH radical scavenging activity was measured according to [7]. Results were expressed as IC_{50} (mg/ml).

Statistical analysis was performed using OriginPro 8 (OriginLab Corporation, Northampton, MA, USA). All data are presented as mean ± standard error of n experiments. Differences were considered statistically significant at $P<0.05$.

Results and discussion

We abbreviated the obtained samples of RC extract with cyclodextrins as follows (Table 1).

Table 1. Abbreviations for cyclodextrin encapsulated forms of RC accepted further in the paper.

Methods	**Samples**	**Abbreviations**
Co-precipitation	RC extract+ Gamma-cyclodextrin	RC/γCD (CP)
	RC extract+2-hydroxypropyl-beta-cyclodextrin	RC/ HPβCD (CP)
Freeze-drying method:	RC extract+ Gamma-cyclodextrin	RC/γCD (FD)
	RC extract+2-hydroxypropyl-beta-cyclodextrin	RC/ HPβCD (FD)
Physical mixture	RC extract+ Gamma-cyclodextrin	RC/γCD (PM)
	RC extract+2-hydroxypropyl-beta-cyclodextrin	RC/ HPβCD (PM)

The total flavonoid content of RC extract and its cyclodextrin encapsulated forms is presented in Table 2. The results demonstrate that TFC in all the samples was lower in comparison to RC extract. The RC/HPβCD (CP) sample revealed the most significant decrease of TFC 58,0±2,75 mg/g DW quercetin eq. Nevertheless it was observed that samples obtained by physical mixture showed higher TFC than by FD and CP methods. Among latter two methods FD method was more efficient than CP method.

Table 2. Total flavonoid content of cyclodextrin encapsulated forms of RC (n=10, mean ± SE)

Samples	**Total flavonoids content (mg/g DW quercetin eq.)**
RC	154,5±14,8
γCD	-
HPβCD	-
RC/γCD (CP)	61,1±0,31
RC/HPβCD (CP)	58,0±2,75
RC/γCD (FD)	64,4±0,85
RC/HPβCD (FD)	63,4±1,85
RC/γCD (PM)	85,1±0,79
RC/HPβCD (PM)	82,6±7,08

The antioxidant research found that CDs encapsulated form of RC extract reveal improved DPPH scavenging activity regardless of the method used. On the other hand chelating activity decrease was observed for all the samples regardless of the method and CD used (Table 3).

Table 3. Antioxidant activity of cyclodextrin encapsulated forms of RC (n=10, mean ± SE)

Samples	**FRAP assay (mg/ ascorbic acid equivalent dry weight)**	**Phosphomolybdenum assay (mg/ ascorbic acid equivalent dry weight)**	**DPPH (mg/ml, IC_{50})**
RC	0,934±0,746	4,05±0,00	8,236±0.07
γ-CD	-	-	-
HP-β-CD	-	-	-
RC/γCD (CE)	0,162±0,004	3,95±0,00	3,965±0.02
RC/HPβCD (CE)	0,511±0,0085	2,48±0,00	2,923±0.00
RC/γCD (LD)	0,075±0,015	3,46±0,00	3,031±0,01
RC/HPβCD (LD)	0,436±0,006	2,49±0,00	2,606±0,01
RC/γCD (PM)	0,480±0,015	2,65±0,00	2,058±0.00
RC/HPβCD (PM)	0,192±0,002	2,74±0,00	1,974±0.00

The antioxidant efficiency of RC extract encapsulated to CDs was found to be dependent on the type of CD and the methods for obtaining encapsulated forms. The encapsulated form of RC with HPβCD obtained by Physical mixture showed improved DPPH radical scavenging activity. Moreover, the formation of CD-antioxidant complexes can lead to a synergistic effect, where the antioxidant activity of the encapsulated compound is further enhanced by the presence of the CD. Despite the promising results obtained so far, further research is needed to fully understand the mechanisms underlying the enhancement of antioxidant activity through CD encapsulation. Moreover, the development of novel CD derivatives and the optimization of encapsulation conditions are crucial for the successful application of this strategy in various fields.

Assuming that oxo and hydroxyl groups of flavonoids are main sites for metal ion chelating the observed results may indicate the complexation between flavonoids of extracts and cyclodextrins. Due to bibliography the formation of inclusion complexes between flavonoids and CDs are likely by insertion of the B-ring of the flavonoids into the cavity of CD. Thus might lead to capping the metal-binding site and decrease in chelating activity. However the hydroxyl at C-6 are considered as essential group for antioxidative activity in the DPPH assay. In this experiment, the increase in DPPH scavenging activity supports the propose of flavonoid-CD inclusion complex formation. Additionally, the formation of CD inclusion complex is possible between other phytocompounds of extract. Further research is necessary to fully understand the mechanisms underlying the enhancement of antioxidant activity through CD encapsulation, despite the promising results obtained so far.

Conclusion

Cyclodextrin encapsulation is a promising approach for enhancing the antioxidant activity of promising herbal plant extracts. The improved stability and increased solubility of the encapsulated antioxidants could lead to the development of more effective antioxidant formulations for various applications, including functional foods, dietary supplements, and pharmaceuticals.

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