

# Development of an Experimental Home Bleaching Agent Containing Chitosan Nanoparticles Loaded with Gypsophila arrostii Extract

Gypsophila Arrostii Ekstresi Yüklü Kitosan Nanopartikülleri İçeren Deneysel Ev Tipi Beyazlatma Ajanının Geliştirilmesi

Selinsu Öztürk<sup>1</sup>, Pınar Yılmaz Atalı<sup>2,3</sup>, Bahar Gök<sup>4,5</sup>, Yasemin Budama-Kılınç<sup>3,4</sup>, Elif Alkan<sup>2,3</sup>, Dilek Tağtekin<sup>2,3</sup>, Şeyma Ulusoy<sup>6</sup>, Murat Kartal<sup>6,7</sup>

<sup>1</sup> Marmara University Institute of Health Sciences, Department of Restorative Dentistry, Istanbul, Türkiye.

<sup>2</sup> Marmara University Faculty of Dentistry, Department of Restorative Dentistry, Istanbul, Türkiye.

<sup>3</sup> Health Biotechnology Joint Research and Application Center of Excellence, Istanbul Türkiye.

<sup>4</sup> Yıldız Technical University Faculty of Chemical and Metallurgical Engineering, Bioengineering Department, Istanbul, Türkiye.

<sup>5</sup> Istanbul Technical University, ARI Teknokent, Raymare Biotechnology Inc., Istanbul, Türkiye.

<sup>6</sup> Department of Pharmacognosy, Faculty of Pharmacy, Bezmialem Vakıf University, Istanbul, Türkiye.

<sup>7</sup> Phytotherapy Research Center, Bezmialem Vakıf University, Istanbul, Türkiye.

## ABSTRACT

**Objectives:** Peroxide alternative agents are currently being developed to provide a safe and effective tooth whitening treatment, given the potential for these agents to affect hard dental tissue. In this study, it is aimed to produce an experimental home whitening gel containing Gypsophila arrostii extract (GE) loaded chitosan nanoparticles with controlled release property.

**Materials and Methods:** GE was prepared in two different ways using water or ethanol, and the saponin amounts of both extracts were measured using gravimetric analysis methods. GE-loaded chitosan nanoparticles (GECNPs) prepared by the ionic gelation method were investigated in terms of size, zeta potential, polydispersity index (Pdl), encapsulation efficiency, and release profile. The significance level was set at  $p < 0.05$ .

**Results:** The water-based extract was preferred for encapsulation due to its statistically significantly higher percentage of saponin content ( $45.38 \pm 3.050396\%$ ) ( $p=0.335$ ). According to the characterization results, GECNPs were  $651 \pm 8.34$  nm in average particle size,  $0.242 \pm 0.02$  Pdl, and  $44.7 \pm 0.21$  mV zeta potential. Encapsulation efficiency was determined as  $97.41 \pm 3.02\%$ . GE release from GECNP was calculated as  $94.11 \pm 4.03\%$  at 48 h, and the drug release rate increased by adding peroxides.

**Conclusions:** The controlled release system of Gypsophila arrostii extract-loaded CNPs was successfully synthesized to develop an experimental home-bleaching gel as an alternative and promising approach.

**Keywords:** Gypsophila arrostii, saponin, chitosan, controlled release system, tooth bleaching

## ÖZ

**Amaç:** Diş sert dokusu üzerindeki potansiyel yan etkileri nedeniyle sağlıklı ve etkili diş beyazlatma tedavisi için peroksit alternatif ajanlar geliştirilmektedir. Bu çalışmanın amacı kontrollü salım özelliğine sahip çöven otu (Gypsophila arrostii) ekstresi yüklü kitosan nanopartikülleri içeren deneysel ev tipi beyazlatma jeli oluşturmaktır.

**Gereç ve Yöntemler:** Gypsophila arrostii ekstresi (GE) su veya etanol kullanılarak iki farklı şekilde hazırlanmıştır ve her iki ekstrenin saponin miktarı gravimetrik analiz yöntemleri kullanılarak ölçülmüştür. İyonik jelleşme yöntemi kullanılarak hazırlanan GE yüklü kitosan nanopartikülleri; partikül boyutu, zeta potansiyeli, polidispersite indeksi (Pdl), enkapsülasyon etkinliği ve salım profili açısından incelenmiştir.

**Bulgular:** Su bazlı GE, istatistiksel olarak anlamlı derecede daha yüksek saponin içeriği ( $45,38 \pm 3,050396$ ) ( $p=0,335$ ) nedeniyle enkapsülasyon için tercih edilmiştir. GE yüklü kitosan nanopartiküllerinin ortalama boyutu  $651 \pm 8,34$  nm, Pdl değeri  $0,242 \pm 0,02$  ve zeta potansiyeli  $44,7 \pm 0,21$  mV olarak ölçülmüştür. Enkapsülasyon etkinliği  $97,41 \pm 3,02$  olarak belirlenmiştir. GECNP'den GE salımının 48 saatte  $94,11 \pm 4,03$ 'e ulaştığı ve salım hızının peroksitlerin ilavesiyle arttığı gözlemlenmiştir.

**Sonuç:** Kontrollü salım sistemi özellikli GE yüklü CNP'lerin sentezi, gelecek vaat eden alternatif bir yaklaşım olarak deneysel bir ev tipi beyazlatma jeli geliştirmek amacı başarı ile gerçekleştirilmiştir.

**Anahtar Kelimeler:** Çöven otu, saponin, kitosan, kontrollü salım sistemi, diş beyazlatma

## Corresponding Author

Selinsu Öztürk (✉)  
selinsuozturk@marun.edu.tr

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## INTRODUCTION

Tooth bleaching, a well-established and effective aesthetic procedure in dentistry, provides a more conservative approach to whitening vital teeth than treatments such as crowns or ceramic veneers (Meireles et al., 2008). The most commonly used components are carbamide peroxide (CP) and hydrogen peroxide (HP), each with specific concentrations for different techniques. The treatment can be performed either in the office or at home (Haywood, 1997). In recent years, home bleaching has become increasingly popular among whitening therapies due to the reduced time spent in the clinic compared to office-based treatments and its lower peroxide content, offering an effective whitening with long-term permanence of the color after the treatment. The most prevalent method of application remains the use of hydrogen peroxide gels with a concentration of up to 6% or carbamide peroxide gels with a concentration of up to 16% for approximately two weeks with a custom tray under the supervision of a dentist (De Geus et al., 2016). Despite its popularity, some adverse effects have been documented. These include tooth sensitivity, decreased bond strength and micro-hardness values, a slight change in surface roughness, and alterations in enamel and dentin mineral content. Although these alterations are regarded as insignificant or reversible, the treatment has been demonstrated to diminish the concentration of calcium and phosphorus within the tooth structure (Pini et al., 2022). Furthermore, carboxy polymethylene, or carbopol, which is frequently utilized as a carrier in CP gels, has also been demonstrated to reduce enamel microhardness (Rodrigues et al., 2005). It would be advantageous to develop a more efficient tooth bleaching method that causes minimal side effects on the enamel, pulp, and oral mucosa while avoiding contraindications. Nowadays, there is a growing trend to turn to natural materials for teeth whitening, as seen in many other areas, and the literature discusses several natural alternatives to peroxide bleaching that can produce oxidative reactions and effectively remove stains without adverse effects (Gopinath et al., 2013; Münchow et al., 2016). Therefore, developing new tooth-bleaching agents based on natural products with minor adverse effects and comparable esthetic results would be beneficial.

Saponins are non-volatile, surface-active compounds that are distributed in nature and are primarily found in the plant kingdom (Oleszek, 2002). Due to their foaming properties, saponins are employed in manufacturing cosmetics, detergents, and soaps. Saponins are also responsible for several other significant functions, including providing antimicrobial, antioxidant, and analgesic properties. A diet high in saponins has been demonstrated to be an effective method for preventing dental caries ((Jyothi & Seshagiri, 2012; Kareem et al., 2022; Timilsena et al., 2023). In addition, saponins derived from various plant sources have been widely used in herbal toothpaste formulations. Research has shown that their antibacterial and antiplaque effects, combined with their ability to promote oral hygiene, make them valuable ingredients in the prevention of dental issues.

Several studies have explored the potential of saponins in whitening teeth and preventing cavities, making them a natural and effective choice for those seeking alternative oral care solutions (Oluwasina et al., 2019; Oluwasina et al., 2023; Paul et al., 2020; Sugianti, 2012).

*Gypsophila arrostii* Guss., a member of the *Gypsophila* genus within the Caryophyllaceae family, is renowned for its triterpene saponins, which are present in its roots. Its notable phytochemical content makes it a promising subject for further study. (Davis et al., 1984); (Arslan et al., 2013). Gypsogenin represents the most prevalent basic structure of saponins isolated from the *Gypsophila* genus. Additionally, quillaic acid and gypsogenic acid are also present in lower concentrations (Arslan et al., 2013). *G. arrostii* is one of the five most utilized *Gypsophila* species in Türkiye (Baytop, 1999). It has been traditionally employed for its diuretic and expectorant activities (Özçelik & Yıldırım, 2011). It is a significant species in the pharmaceutical industry, serving as the primary source of saponins in vaccine adjuvants (Arslan & Cenzano, 2021). Moreover, *G. arrostii* is utilized to produce tahini halva, a significant ingredient in the Turkish food industry (Arslan, 2017). It serves as an emulsifier, facilitating the combination of sugar and sesame oil, bleaching the product's color, and enhancing its volume. Additionally, gypsophila species are employed in the cosmetic industry due to their gentle cleansing, soothing, and anti-inflammatory properties. Furthermore, they are known to enhance the penetration of other skincare ingredients (Bezerra et al., 2018). Additionally, reports indicate that the species is used in bleaching agents due to its saponin content (Guo et al., 2022). The synergistic effect of saponin compounds and their free radical neutralizing activity may play a significant role in their bleaching properties (Kang et al., 2024). An in vitro study investigating the effects of *Hibiscus Sabdariffa* extract on discolored teeth demonstrated that its saponin content contributed to whitening efficacy (Sugianti, 2012). Based on these findings, the saponin content of *G. Arrostii* extract was utilized to evaluate its potential bleaching properties in the present study. Nevertheless, there is a lack of studies in the literature evaluating the potential use of the *Gypsophila* genus in dentistry.

Chitosan, a natural polymer produced due to the deacetylation of chitin obtained from the shell of arthropods, acts as a thickener and carrier with its film-forming properties. It is known to protect the tooth from erosion and demineralization by acting as a stable barrier under enamel or dentin (Guo & Gemeinhart, 2008). Due to its biocompatible, bioadhesive, bioactive, and non-toxic properties, chitosan is frequently preferred in nanoparticle production (Peniche & Peniche, 2011). Nanoparticles (NPs) formed with chitosan with ionic cross-link structure have the advantages of prolonged release of drugs, protection against enzymatic degradation, and biodegradability (Sun et al., 2010). Concerns regarding the biocompatibility and toxicity of natural compounds present a significant challenge in their use; however, encapsulating plant compounds in polymeric nanoparticles helps to minimize potential adverse effects and increase bioavailability

(Patra et al., 2018). Encapsulation protects sensitive components and ensures a controlled release of active substances. Controlled drug release ensures that the encapsulated drug is delivered to the correct site, maintaining the appropriate amount of the drug in the target tissue while minimizing adverse side effects. Controlled release systems offer a number of advantages, including enhanced efficacy, high stability against enzymatic and other forms of degradation of encapsulated or immobilized components, reduced toxicity, and simplified application (Aydın & Ünlüel, 2021). Considering all this information, in the present study, experimental bleaching gels containing a controlled release system of chitosan loaded with *G. arrostii* extract (GE) were developed to minimize the potential side effects of whitening treatment and to provide an effective and stable whitening. The ionic gelation method was used in the synthesis of GE-loaded chitosan nanoparticles (GECNPs). Encapsulation capacity is essential for effective and long-term release in controlled release systems (Egil et al., 2020). Therefore, the encapsulation efficiency (EE) of GECNPs was determined in our study. The average particle size (Z-Ave), multiple distribution index (Pdl), and Zeta potential (ZP) values were analyzed, and the optimum nanoparticle was determined. *In vitro* release properties were also investigated.

## MATERIALS AND METHODS

The materials utilized in the study are presented in Table 1.

**Table 1.** Materials used in the study

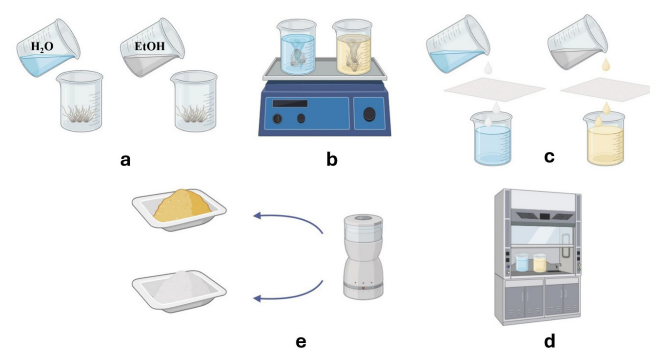
Material	CAS no.	Manufacturer
Chitosan (75-85% deacetylated, low molecular weight)	9012-76-4	Sigma-Aldrich (St. Louis, MO, USA)
Sodium tripolyphosphate	7758-29-4	
Diethyl ether	60-29-7	
Ethanol	64-17-5	
<i>n</i> -Butanol	71-36-3	
Sodium chloride	7647-14-5	
Hydrogen peroxide (30%)	7722-84-1	
Carbamide peroxide (97%)	124-43-6	Merck
Acetic acid	64-19-7	
<i>G. arrostii</i> roots*	-	Fitovizyon Doğal ve Sağlıklı Yaşam Ind. Trade Co. Ltd. (Istanbul, Türkiye)

\**G. arrostii* which is one of the five widely distributed and most used *Gypsophila* species in Türkiye, was purchased from Fitovizyon Doğal ve Sağlıklı Yaşam Ind. Trade Co. Ltd. (Istanbul, Türkiye) and identified by Prof. Dr. Murat Kartal. A voucher specimen was deposited at the Bezmialem Phytotherapy Research Center.

### Preparation of GE

Two different extracts were prepared using the roots of *G. arrostii*. In Beaker A, 600 mL of water was added to 150 g of *G. arrostii* root. In Beaker B, 600 mL of 50% ethanol (EtOH) was added to 150 g of *G. arrostii* root. The two different mixtures were stirred in a magnetic stirrer at 50

°C, and 200 rpm for 4 hours. The extracts were filtered through filter paper and kept in a fume hood until dry. The dry extracts were ground with a grinder to a fine powder (Fig. 1).



**Figure 1.** Preparation of GE

a. Preparation of beakers, b. Stirring in a magnetic stirrer, c. Filtering through filter paper, d. Drying in fume hood, e. Grinding with a grinder

### Saponin Measurement

The saponin content was determined using gravimetric methods (Arwani et al., 2019). 20 mL of 20% ethanol was added to 2 g of dry *G. arrostii* extract in a conical flask. 10 mL diethyl ether was added to the concentration in a 100 mL separatory funnel and shaken vigorously, from which the ether layer was discarded, and the aqueous layer was recovered. 20 mL *n*-butanol was added and extracted twice with 5 mL of 5% sodium chloride. After removing the sodium chloride layer, the remaining solution was heated in a water bath for 30 min, transferred into a beaker, and then dried in an oven to a constant weight. The saponin content was calculated as a percentage:

### Spectrophotometric Analysis of GE

Seven distinct concentrations (7.8125, 15.625, 31.25, 62.5, 125, 250, and 500 µg/mL) were prepared, and their absorbance values were measured with a UV spectrophotometer to obtain a calibration curve which was subsequently utilized to determine the EE and analyze the *in vitro* release profile.

### Preparation of GECNPs

Synthesis of GECNPs was carried out using the ionic gelation technique. Chitosan (3% w/v) was dissolved in water containing 1.5% (v/v) acetic acid solution and stirred on a magnetic stirrer (Daihan-Digital Magnetic Stirrer, Korea) for 24 h. 25 mg of sodium tripolyphosphate (TPP) was dissolved in 50 mL of distilled water. 10 mg of dry extract was dissolved in 1 mL of distilled water and added to the TPP solution. Then, after stirring at 250 rpm, it was mixed with chitosan solution and sonicated with 70 W energy for 5 minutes (Kaymak et al., 2024).

## Preparation of Peroxide Based Experimental Bleaching Gels

100 mL solution of *Gypsophila* extract-loaded chitosan nanoparticles were mixed with HP at specified proportions to obtain a 6% HP experimental bleaching gel, while CP was mixed at specified proportions to obtain a 16% CP experimental bleaching gel.

## Dynamic Light Scattering (DLS) Analysis

The Zetasizer Nano ZS (Malvern Instruments, England) device was used to measure Z-Ave, Pdl, and ZP of the produced chitosan-controlled release system. The particles were placed in a transparent cuvette, and measurements were taken with a 4.0 mV He-Ne laser (633 nm) at 25°C. Each sample was prepared by diluting it with water at a ratio of 1:10. The Z-Ave, Pdl, and ZP values of the samples were calculated by taking the average of three measurements.

## Encapsulation Efficiency (EE)

5 mg of GECNPs were dissolved in 10 mL ethanol and sonicated in an ultrasonic bath for 30min. The quantity of encapsulated GE was calculated from the absorbance value obtained from the calibration curve using Equation 1.

$$\text{Encapsulation efficiency(\%)} = \frac{\text{Total amount of loaded GE}}{\text{Initial amount of loaded GE}} \times 100 \quad (1)$$

## In vitro Release Profile

The *in vitro* release profile of GECNPs, GECNPs-HP, GECNPs-CP, and GE (as a control group) was determined using the membrane diffusion technique. A total of 1 mg of GECNPs were suspended in 1 mL of distilled water and placed on dialysis membranes. The release was conducted in 100 mL of PBS (pH 6.5) in a shaking water bath set at 25 °C room temperature at 120 rpm. At the designated time intervals (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 24, and 48 h), 1 mL sample was taken from the release medium and replaced with an equal volume of buffer to maintain a constant volume in the release medium. Measurements were performed with a UV-Vis spectrophotometer (Shimadzu, UV-1280, Japan), and the amount of GE in the sample was calculated according to the standard curve Equation 2.

$$\text{Release (\%)} = \frac{\text{Released amount of GE}}{\text{Total amount of GE}} \times 100 \quad (2)$$

## Statistical Analysis

Data were analyzed with IBM SPSS v23, using Shapiro-Wilk and Independent sample t-test; the significance level was taken as  $p < 0.05$ . Additionally, the results are expressed as the mean  $\pm$  standard deviation derived from three independent measurements.

## RESULTS

### Gravimetric Total Saponin Determination

The total weights of the *G. arrostii* root extracts prepared with 50% ethanol and 100% water were 45.6000 g and 42.8320 g after drying. Percentage of total saponin amounts determined gravimetrically from 2 g samples ( $n=3$ ) of dried extracts are given in Table 2. A statistically significant difference was observed between ethanol and water-based extracts (0,035). The mean saponin content of the GEs prepared using ethanol was determined to be  $39.83 \% \pm 0.2211$ , whereas the GEs produced with water exhibited a higher saponin concentration of  $45.38 \% \pm 3.0504$ . According to these results, based on the saponin yield in dried 50% ethanol and 100% water extracts, the saponin yields of the *G. arrostii* roots were calculated as 12.1083 % and 12.9581 %, respectively.

**Table 2.** Percentage of total saponin amounts

Preparation conditions	n	g/2g	%	Mean % ± SD	P
50 % EtOH	1	0.8000	40.0000	39.83 % ± 0.2211	0,035
	2	0.7916	39.5800		
	3	0.7982	39.9100		
100 % H <sub>2</sub> O	1	0.9715	48.5750	45.38 % ± 3.0504	
	2	0.8499	42.4975		
	3	0.9015	45.0750		

## Nanoparticle Characterization

### Evaluation of Average Size, Polydispersity Index, and Zeta Potential

In this study, physicochemical properties such as Z-Ave, Pdl, and ZP value of GECNPs were determined according to DLS analysis. The results of the blank CNPs and GECNPs are presented in Table 3. According to the results, the blank chitosan-controlled release system has a Z-Ave of  $1499 \pm 46.67$  nm, Pdl of  $0.186 \pm 0.12$ , and ZP value of  $43.8 \pm 1.56$  mV. GECNPs have a Z-Ave of  $651.3 \pm 8.34$  nm, a Pdl of  $0.242 \pm 0.02$ , and a ZP value of  $44.7 \pm 0.21$  mV.

**Table 3.** Dynamic light scattering analysis results of blank CNPs and GE-CNPs

Samples	Average Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
Blank CNPs	$1499 \pm 46.67$	$0.186 \pm 0.12$	$43.8 \pm 1.56$
GECNPs	$651.3 \pm 8.34$	$0.242 \pm 0.02$	$44.7 \pm 0.21$

## Encapsulation Efficiency

The EE of GECNPs was calculated as  $97.41\% \pm 3.02$  using the calibration curve given in Fig. 2.



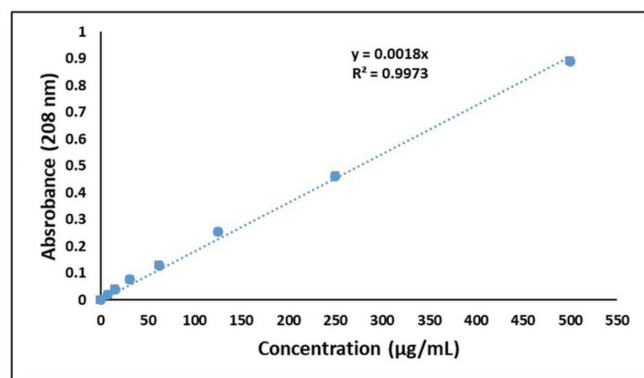


Figure 2. The calibration curve of GE.

### *In vitro* release study

The *in vitro* release graph of GECNPs was plotted as time and % amount of release (Fig. 3). The experimental groups demonstrated varying release profiles. GE release from GECNP reached  $94.11 \% \pm 4.03$  at 48 hours, while in the GECNP - 6%  $H_2O_2$  group, it was  $89.83 \% \pm 2.01$  at 3 hours, and in the GECNP-16% CP group, it was  $88.40 \% \pm 4.30$  at 24 h. In comparison, the release of free GE was nearly complete ( $92.68 \% \pm 2.05$ ) within 5 h.

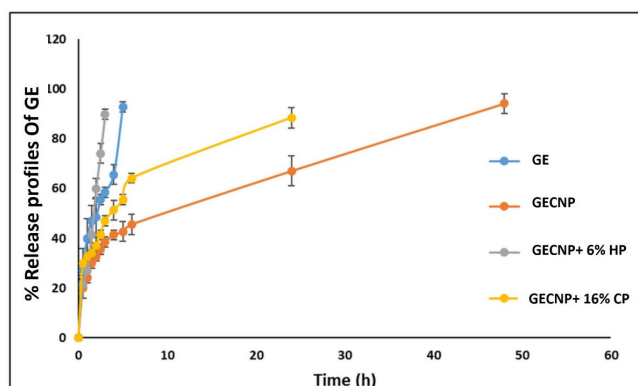


Figure 3. Controlled release graph of GE, GECNPs, and GECNPs with different components (HP, CP)

## DISCUSSION

Nanoparticles can enhance product quality by incorporating various functional groups. As a result, nano products are extensively utilized in different industrial sectors, including medicine and dentistry. Chitosan exhibits distinctive characteristics, including biocompatibility, biodegradability, antimicrobial properties, and analgesic effects (Arnaud et al., 2010).

Chitosan nanoparticles prepared with TPP offer significant stability and don't require the use of external stabilizing agents or cross-linkers (Khan et al., 2016). Chitosan has already been incorporated into bleaching gels and has demonstrated no interference with their whitening effectiveness (Kolsuz Ozcetin & Surmelioglu, 2020; Pini et al., 2022). However, its potential to reduce the side effects of bleaching remains uncertain. Plant compounds

are encapsulated in polymeric nanoparticles to minimize potential side effects (Patra et al., 2018). In the light of all this information, chitosan was used to encapsulate GE and increase its bioavailability. In the present study, it was decided to utilize *G. arrostii* due to its high saponin content and obtain concentrated dried extracts with even higher saponin contents. Previously published studies have revealed that the roots of *G. arrostii* contain 11-22 % of total saponins (Battal et al., 2003; Koyuncu et al., 2008). According to the findings of our study, based on the saponin yield in dried 50 % EtOH and 100 %  $H_2O$  extracts, the saponin yields of the *G. arrostii* roots were calculated as 12.1083 % and 12.9581 %, respectively, which makes apparent that the roots we have used meet the criterion of having saponin 11-22 %. When we calculated the percentage of saponin content for both the 50% ethanol-based and the water-based dried and concentrated extracts, as presented in Table 2, the water-based extract was preferred for encapsulation due to its statistically significantly higher percentage of saponin content ( $45.3800 \pm 3.0504\%$ ) ( $p=0.035$ ).

Dynamic light scattering is commonly used to determine the hydrodynamic dimensions of nanoparticles, providing various advantages in nanoparticle sizing (Egil et al., 2020). In our study, Z-Ave, PDI, and ZP values of GECNPs were measured based on DLS principles. According to the results in Table 3, CNPs have a 1499 nm Z-Ave, 0.186 PDI, and 43.8 mV ZP value. However, GECNPs had a 651.3 nm Z-Ave, 0.242 PDI, and 44.7 mV ZP value (Table 3). According to the findings, loading CNPs with the extract decreased the Z-Ave, and increased the PDI, and the ZP value. Various parameters, including the synthesis conditions, the component mixing ratios, and the densities of extracts affect the size of the NPs (Egil et al., 2020). Weak and/or electrostatic interactions between the polymer and the plant extract chemical groups may contribute to the size variations seen in drug-loaded NPs (Manne et al., 2020). In the present study, the increase in particle size is attributed to the high chitosan content, while the inclusion of GE leads to a decrease in the size of the NPs. NPs are defined as particles smaller than 100 nm (Borm et al., 2006; Dowling, 2004). However, the size of polymeric NPs can range from 10 to 1.000 nm, making them suitable for a variety of applications (Gheffar et al., 2021; Hamzaoui & Laraba-Djebbari, 2021; Ni et al., 2021; Roberts et al., 2020). The PDI value reflects the homogeneity of nanoparticle size, with a PDI value of 0.2 or lower indicating a more uniform distribution of nanoparticles (Rajamanickam & Manju, 2023). Neuroprotective flavonoid-loaded CNPs synthesized by Soltanzadeh et al. and myricetin-loaded chitosan nanoformulations synthesized by Upadhyay et al. had PDI values of  $0.260 \pm 0.015$  and  $0.272 \pm 0.02$  respectively (Soltanzadeh et al., 2021; Upadhyay et al., 2023). While the blank CNPs obtained in our study showed a uniform distribution, GECNPs demonstrated similarities with findings reported in the literature. The zeta potential allows us to assess the surface charge and stability of a nanostructure; ZP values greater than  $\pm 30$  mV indicate a high level of stability in the nanoparticles (Simunkova et al., 2009). Based on our results, the ZP value of the

synthesized empty CNPs and GECNPs was above +30 mV, demonstrating good stability. The slight increase in the ZP value of GECNPs may be ascribed to the plant extract enhancing the number of positively charged groups on the surface of the nanoparticles (Manne et al., 2020).

Encapsulation is an essential strategy for stabilizing molecules and enhancing their efficacy. Hence, it is crucial to calculate and measure EE when preparing NPs (Shen et al., 2017). In the present study, the EE value was  $97.41 \pm 3.02\%$ . This value is in accordance with those reported in previous studies. Egil et al. prepared Epilobium extract (EPE) loaded chitosan nanoparticles, reporting an EE of 92.46% (Egil et al., 2020). Similarly, Khan et al. synthesized chitosan nanoparticles loaded with Curcumin, indicating that the EE was determined to be 85% (Khan et al., 2016).

*In vitro* release kinetics are critical as they indicate a molecule's pharmacokinetic and pharmacological behavior *in vivo* (Abdelkader et al., 2020). *In vitro* release studies were conducted using the membrane diffusion technique and monitored for 48 h, pH adjusted 6.5 to mimic the *vivo* conditions in the oral cavity accurately. In the present study,  $94.11 \pm 4.03$  of the free GE loading was released from GECNPs within 48 hours. Similarly, Egil et al. reported that 96.04 % of EPE was released at pH 6.5 within the same 48-hour period (Egil et al., 2020). In the peroxide-based groups (GECNP-HP, GECNP-CP), it was found that the addition of peroxides accelerates GE release from GE-CNPs. In the presence of 6% HP and 16% CP, the release was  $89.83 \pm 2.01\%$  at 3 hours and  $88.40 \pm 4.30\%$  at 24 hours, respectively. This acceleration may be attributed to the acidic pH of HP and CP, as well as the pH-responsive properties of chitosan under slightly acidic conditions.

The European Scientific Committee on Consumer Products (SCCP) has indicated that tooth bleaching agents containing hydrogen peroxide ranging from 0.1% to 6.0% or equal hydrogen peroxide-releasing agents are considered safe when used under the guidance of a dentist (Affairs, 2008). Home bleaching gels can be applied to the enamel surface for a maximum of 1.5 and 6 hours, respectively, depending on the  $H_2O_2$  or CP concentration they contain, and it is known that the effectiveness of the gels continues within the tooth structure even after the application period. So, accelerated extract release can be considered an advantage of whitening procedures.

## CONCLUSION

In accordance with the *in vitro* findings, the GECNP formulation exhibited favourable physicochemical characteristics with regard to particle size, PDI, and zeta potential. GE was successfully encapsulated within CNPs. The incorporation of peroxides resulted in an accelerated release profile. Consequently, the developed controlled release system of GECNPs represents a promising potential agent for tooth bleaching. However, further studies are necessary to evaluate the potential cytotoxicity and assess its effects on dental hard tissues.

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## Conflicts of Interest

The authors have no financial interest in any companies or products mentioned in this article.

## Ethical Approval

Not applicable.

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