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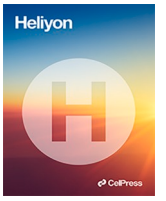

Research article

# Semi-quantitative analysis on sea buckthorn phenolic-rich extract coating bone-like open porous NiTi-based alloy

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ABSTRACT

This study investigates the feasibility of coating Ni–Ti alloy with sea buckthorn extract via a hydrothermal method for targeted delivery of beneficial phenolic compounds to bone tissue. The qualitative analysis confirmed the presence of flavonoids and tannins in sea buckthorn extract, supporting its osteogenic potential. The microhardness of the NiTi alloy substrate was suitable for biomedical applications, and successful coating was achieved without compromising its properties. NiTi alloy samples were coated with 18.1, 20.1, and 12.4 mg of extract, respectively. Comprehensive evaluations confirmed the successful integration of the extract onto the alloy's surface. The coated system exhibited sustained release properties over five days, with the highest release occurring on the first day (on average 32.1 % for the first peak and 72.1 % for the second peak), as determined by HPLC analysis. The findings demonstrate the potential of this novel approach in developing dual-functionality implants for bone health promotion. Overall, this study underscores the promising potential of Ni–Ti alloy coated with sea buckthorn extract as a targeted drug delivery system for bone tissue.

## 1. Introduction

*Elaeagnus rhamnoides* (L.) A. Nelson, belonging to the Elaeagnaceae family, is a plant species that grows naturally in Bulgaria, Iran, Turkey, and the Caucasus regions. It is commonly known as "Sea buckthorn," its scientific synonym is *Hippophae rhamnoides* L. The plant is typically found on the sandy banks of rivers and roads in Anatolia and is also cultivated as a hedge along the borders of fields. The plant's fruits are used for their constipation-relieving, antiseptic, and strengthening properties. Due to their high vitamin C content, the fruits are also used in Turkish folk medicine to combat colds and flu [1].

Previous research has highlighted the plant's numerous beneficial effects, including its antitumor, antioxidant, antimutagenic, and antibacterial properties. The plant has also been effective in treating various health conditions such as atopic dermatitis,

*Abbreviations:* HPLC, high-performance liquid chromatography; Niti, nickel-titanium alloy.

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cardiovascular diseases, liver damage, gastric ulcers, and burns [2]. A recent study revealed that feeding mice fruits from the plant prevented bone loss after undergoing ovariectomy [3]. An alcohol extract of *E. rhamnoides* rich in flavonoids was found to shield bone marrow cells from radiation damage and hasten their recovery [4]. Recent meta-analyses have consolidated data on *E. rhamnoides*, affirming its efficacy in managing chronic diseases and highlighting its potential as a natural adjunct therapy.

Bone formation, also known as osteogenesis or bone ossification, begins during embryonic development and continues until around age 25, though this can vary. There are two types of ossification: intramembranous and endochondral. While both start with a mesenchymal tissue precursor, they differ in how this precursor transforms into bone. Intramembranous ossification directly converts the mesenchymal tissue to bone and creates the flat bones of the skull, clavicle, and most of the cranial bones, while endochondral ossification involves the mesenchymal tissue transforming into a cartilage intermediate, which later becomes bone and forms the remaining axial skeleton and long bones. Research has increasingly focused on natural compounds that can synergistically support osteogenesis, with *E. rhamnoides* extracts showing promise in stimulating osteoblastic activity and mineral deposition *in vitro*.

Osteoblasts play a vital role in the formation of bones by depositing new bone tissue and regulating the activity of osteoclasts. These specialized cells originate from mesenchymal stem cells and secrete an unmineralized matrix called osteoid during the embryonic period. This matrix then undergoes calcification to become a fully formed bone. By maintaining the balance between bone formation and resorption, osteoblasts ensure the structural integrity and strength of the skeletal system. Multinucleated cells known as osteoclasts play a crucial role in bone resorption [5]. Osteoclasts originate from precursor cells that are related to macrophages, and they migrate into bone tissue through blood vessels [6,7]. Osteocytes, which are the most abundant cells found in bone tissue, arise from osteoblasts that become embedded in the mineralized matrix known as osteoid [8]. The main role of osteocytes is to serve as mechanosensors within the bone tissue. These cells are equipped with cytoplasmic processes that allow them to establish connections with both neighboring cells and the extracellular environment, which enables them to detect and respond to mechanical stimuli [9].

Drug delivery systems are designed to improve the efficiency and effectiveness of drug therapies by controlling the release and targeting of drugs to specific sites in the body. Implants are devices that are surgically placed inside the body, and they can be made from a variety of materials, including polymers, metals, and ceramics. They can be used as drug delivery systems to provide sustained release of drugs to targeted areas of the body [10].

Implantable drug delivery systems offer several advantages over traditional drug delivery methods, such as oral tablets and injections. Some of the benefits of using implants as drug delivery systems include: sustained drug release, targeted drug delivery, and improved patient compliance. Implants can be used to deliver a wide range of drugs, including antibiotics, hormones, chemotherapeutic agents, and herbal extracts as in this study [11].

Due to the porous structure of Ni–Ti alloy the material, it has high drug-loading capacity, controlled release, and biocompatibility, making it particularly suited for prolonged therapeutic applications in targeted drug delivery scenarios. NiTi alloy has been used in biomedical implants, including bone implants. NiTi alloy has excellent mechanical properties and biocompatibility. Porosity varies of the NiTi depending on process parameters. Since a uniform production parameter was used in the produced samples, a single porosity value was obtained. The optimum pore range of NiTi alloys produced in the range of 30–90 % porosity is between 100 and 400 μm, which is the most preferred range for bone implant materials. The porosity of the NiTi Alloy we obtained was measured to be approximately 46 %. The pore size was obtained in the range of 200–400 μm for large pores and 25–50 μm for small pores, and these pore sizes are considered to be sufficient for bone implants [12]. NiTi alloy, also known as Nitinol, is renowned for its unique shape memory and superelastic properties, allowing it to return to a predetermined shape when heated above a certain temperature. These characteristics, combined with excellent biocompatibility and corrosion resistance, make Nitinol an ideal material for medical devices, such as stents and orthodontic wires, and various aerospace and automotive applications. NiTi-based shape memory alloys (SMA) with biocompatibility, osseo-integration, and super elasticity, are very similar form to human bone and have mechanical properties close to natural bone structure.

In addition, its porous structure, which allows substance transfer due to its open porous structure, becomes a preferable material for situations where the replacement of human bone is required. Research on the production methods of biomaterials, which have gained many innovations with many studies in the surgical field, has increased at an increasing rate. In particular, the usage areas and commercialization speed of metallic biomaterials used in the body are becoming increasingly widespread. Obtaining a biomaterial with bone-like properties and in the desired optimum range of properties can only be achieved through interdisciplinary studies. Many methods have been used in the production of porous NiTi SMA. List them, hot isotactic pressing (HIP) [10–12], metal injection molding method (MIM) [10], spark plasma sintering method (SPS) [13], vacuum induction melting technique (VIM) [14], self-leveling high-temperature synthesis (SHS) [15–18] and conventional sintering methods. The SHS method, self-leveling high-temperature synthesis, is defined by its unique superior properties. Self-propagating high-temperature synthesis (SHS) is one of the techniques developed to design and develop porous alloys, ceramics, and various intermetallic compounds from a compressed powder mixture in the SHS method, titanium, aluminum, etc. It starts with igniting a mixture of reactive powders and a non-reactive powder. SHS is a technique for creating porous alloys, ceramics, and various intermetallic compounds from a compacted powder mixture, including elements like titanium and aluminum. This method begins by igniting a mixture of reactive and non-reactive powders. The ignition triggers a series of combustion reactions due to sufficient heat release, transforming the compacted powder into a final product with open pores and notable durability [19]. As a result of the initiation of the ignition process and then sufficient heat release, sequential combustion reactions are initiated. As a result of the SHS process, the compacted powder alloy is converted into a stable and durable final product with open pores.

During the process, the diffusion rate and combustion temperature vary according to stoichiometry, preheating temperature, the reactive particles' size, and the diluent amount [20–23]. Gil et al. produced NiTi and NiTiCu SMAs and investigated the microstructural, mechanical, and cytotoxic properties of the produced SMAs. They compared the Cu-doped NiTi alloys they obtained with

the pure NiTi alloys. They reported that adding Cu was more successful in terms of toxicity and super flexibility properties studied in cells cultured with human fibroblasts [24].

Hafez et al., on the other hand, did not detect any toxicity at the cellular level when titanium or nickel-titanium was used in their study on the cytotoxic effects of NiTi SMAs. The surface properties of NiTi-based SMAs can be further improved with various coatings. Especially by forming thin film coatings on this alloy surface, cell adhesion and reproduction ability can be improved. In addition, the situation of showing toxic properties can be prevented [25].

For this reason, the hydrothermal method was preferred in this study, and NiTi SMAs coated with phenolic-rich extract were formed with this method. Due to the advantages of this method, such as high efficiency, low energy requirement, obtaining the desired composition with high purity, and saving time, NiTi SMAs produced by the SHS method were used. The hydrothermal coating method is both environmentally friendly and non-hazardous, offering a unique blend of high efficiency, environmental friendliness, and excellent biocompatibility compared to other methods, making it an attractive choice for biomedical applications. It reflects a growing trend towards green chemistry approaches in materials science, aiming to reduce environmental impact while enhancing material performance. It also allows obtaining coatings with desired properties by using low temperatures. The selection of phenolic-rich extracts for coating NiTi alloys is informed by their proven antioxidative and anti-inflammatory properties, which are hypothesized to contribute to improved biocompatibility and longevity of biomedical implants and their role in osteogenesis.

In this study, firstly qualitative analysis of secondary metabolites as alkaloids, cardioactive heterosides, flavonoids, anthocyanosides, and tannins. Additionally, the total phenolic content of the aqueous extract of *E. rhamnoides* was determined. Then, Ni–Ti alloy materials were coated with the phenolic-rich extract via the hydrothermal method. The release in PBS day by day of the extract from Ni–Ti alloy material for five days was determined by using HPLC.

## 2. Materials and methods

### 2.1. Plant material

In September 2016, *Elaeagnus rhamnoides* (L) A. Nelson leaves were collected from the province of Askale in the city of Erzurum, Turkiye. The plant material was verified by Mehmet Önal and a voucher sample (No. AUEF 1350) was submitted to the Biodiversity Application and Research Center at Atatürk University, Erzurum, Turkiye.

### 2.2. Extraction

Our previous study provided a detailed account of the leaf extraction process [26]. Specifically, 800 g of dried and powdered leaves were extracted using 70 % methanol. The resulting methanol extract was dissolved in distilled water and fractionated into four sub-fractions using *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol. The remaining water phase and sub-fractions were concentrated, ultimately yielding an aqueous extract weighing 142.2 g.

### 2.3. Qualitative analysis of secondary metabolites

#### 2.3.1. Qualitative analysis of alkaloids

To prepare the samples for analysis, 0.5 g of extracts were boiled in a solution of 10 mL 70 % ethanol containing 6 % $H_2SO_4$ for 1 min. The solution was then allowed to cool and settle. A small amount of the upper liquid was taken from the solution and added to two separate tubes, to which Mayer and Dragendorff reagents were added to check for precipitation.

After confirming the presence of precipitate, the ethanolic extract was transferred to a small separating funnel and alkalized with enough 25 % $Na_2CO_3$ solution. The mixture was then rinsed with 15 mL of chloroform and combined with 15 mL of 10 % acetic acid solution. The resulting mixture was separated into 3 tubes, with one tube kept for control purposes. Mayer reagents were added to the second tube, and Dragendorff reagents were added to the third tube to check for precipitation [27].

#### 2.3.2. Qualitative analysis of cardioactive heteroside

To prepare the samples for analysis, they were boiled in 10 mL of 70 % ethanol in a water bath for 2 min and filtered. The resulting filtrate was then diluted 2 times with water, and 1 mL of concentrated lead subacetate solution was added before filtering again. The filtrate was then extracted using 10 mL of chloroform, and the resulting chloroform phase was separated into 3 capsules for further analysis using the Keller-Kliani and Baljet reactions [27].

#### 2.3.3. Qualitative analysis of flavonoids

Cyanidin reaction: The samples were mixed thoroughly with 5 mL of ethanol and heated gently to extract the compounds. The resulting extract was then filtered, and 0.5 mL of concentrated HCl and a small amount of Mg powder were added to the filtrate. The reaction was observed for any color changes or the formation of foam, indicating hydrogen evolution [27].

#### 2.3.4. Qualitative analysis of anthocyanoside

To extract the compounds from the samples, 50 % ethanol was used over low heat. The resulting extract was filtered, and the filtrate was then divided into five parts to carry out the following reactions.

1. The color formed by the addition of diluted $H_2SO_4$ was observed.
2. First, NaOH solution was added and then the mixture was acidified with HCl, and the resulting colors were observed.
3. The formation of precipitation with 10 % lead acetate solution was observed.
4. A small amount of amyl alcohol was added, and the mixture was shaken to observe the coloration of the layers.
5. The mixture was slightly heated, cooled with diluted $H_2SO_4$, and then rinsed with amyl alcohol. The coloration of the amyl alcohol layer was observed [27].

#### 2.3.5. Qualitative analysis of tannin

To prepare a 5 % infusion from the samples, they were infused and the resulting infusion was used to conduct the following tests.

1. The color formed upon the addition of 5 % $FeCl_3$ was observed.
2. The formation of precipitation with 1 % saline gelatin solution was observed.
3. The formation of precipitation upon the addition of brominated water was observed.
4. The precipitate formed by Stiasny's reagent was observed [27].

### 2.4. Total phenolic content determination

The method developed by Folin & Denis and modified by Slinkard & Singleton was used to determine the total phenolic compound amounts of the aqueous extract. Gallic acid was used as the reference phenolic compound, and gallic acid solutions at concentrations of 100, 200, 300, 400, 500, 600, and 700 μg/ml were treated with Folin-Ciocalteu Reagent (FCR) and aqueous $Na_2CO_3$ to obtain a standard graph. Absorbances were recorded at 760 nm (blank: distilled water), and stock solutions of plant extracts were prepared at a concentration of 1 mg/ml. Samples were treated the same way as standard solutions, and absorbances were recorded at 760 nm using a Thermo Scientific Multiscan Go UV–Vis spectrometer (Thermo Fisher Scientific, California, USA). The gallic acid equivalents corresponding to the absorbance values of the samples were determined using the equation obtained from the reference graph, and the results are reported in gallic acid equivalents (GAE) and micrograms. The assays were conducted in triplicate [28,29].

### 2.5. Coating process of Ni–Ti alloy with extract

Three NiTi-based samples were fixed vertically in the middle of a Teflon Lined reaction vessel. The aqueous extract prepared for hydrothermal synthesis was dissolved in 60 mL of deionized water. Then, the prepared solution was stirred at room temperature for 12 h in a magnetic stirrer, and a dark brown homogeneous solution was obtained. Next, the prepared extract solution was transferred to a 60 mL Teflon-coated autoclave and put into the hydrothermal reactor for hydrothermal synthesis. The hydrothermal synthesis process was carried out in a Fytronix Electronic Company brand PID-controlled hydrothermal device (Fytronix FYHT-8000) at 120 °C for 24 h. After hydrothermal synthesis, the samples were dried in a vacuum oven (HT600, Fytronix) for 24 h at 50 °C under 50 mbar pressure for further characterization.

### 2.6. SEM-EDS analysis

NiTi alloys with a composition of 51.8 % Nickel and 48.2 % Titanium have a structure with wide pores, as shown in Fig. 2. The pore sizes, calculated using ImageJ software, account for 21.08 % of the alloy's total area. The surface morphology of the before and after coated samples was analyzed using a Zeiss-Sigma 300 model scanning electron microscope (SEM) at a voltage of 8 kV for Figs. 3a and 5 kV for Fig. 3b. The chemical compositions of the extract-coated sample were analyzed through the use of an energy dispersive spectrometer (EDS), employing a power of 2.790 keV for Figs. 3c and 0.740 keV for Fig. 3d. The EDS analysis utilized a $Si_3N_4$ (silicon nitride) detector, EDAX Element 2 with 129 eV resolution, and a 30 $mm^2$ detector size, equipped with Peltier cooling. SEM and EDS instruments were accessed in the Ataturk University East Anatolia High Technology Application and Research Center (DAYTAM).

### 2.7. Determination of in-vitro extract release by HPLC

The effects of porous NiTi alloy material on extract release were evaluated for each three samples. The HPLC was used for the semi-quantitative analysis according to peak areas about the release. HPLC was performed using Agilent 1260 liquid chromatography with a diode array detector (DAD) (Agilent Technologies Inc.). Each coated sample was placed into three separate tubes and 10 mL of phosphate-buffered saline (PBS) was added. The tubes were then placed in a shaking water bath (MiproLab) at 36.5 °C and 37 rpm. On the first day, samples were taken during the 1st, 2nd, and 3rd hours. Subsequently, samples were taken on the 1st, 2nd, 3rd, 4th, and 5th days. Each time, the entire solution inside the tube was emptied, and 10 mL of fresh PBS was added. Each solution was then transferred to vials and analyzed by HPLC. The HPLC profile was compared to that of the extract prepared at a concentration of 1 mg/mL (10 mg extract was dissolved in 10 mL PBS) [30].

Supelco Ascentis Express RP-Amide column (100 × 2.1 mm, 2.7 μm) was used as the stationary phase. The column temperature was maintained at 40 °C. The mobile phase consisted of a gradient system of ACN:0.2 % FA (10:90 → 45:55, v/v) at a flow rate of 0.3 mL/min and a run time of 32 min. The injection volume was 20 μL. The absorbance of the eluent was monitored at 254 nm. Each sample was analyzed in triplicate. Two peaks as compounds were seen on the HPLC chromatogram (Rt 1st peak: 18.263 min, Rt 2nd peak: 20.192). The peak areas were presented as means ± standard deviation.

## 3. Results and discussion

Our study focused on investigating the coating ability by the hydrothermal method using sea buckthorn extract, with a single production parameter emphasizing the mechanical integrity of the implant for its intended biomedical application.

The phenolic compounds in the extract coating under physiological conditions were assessed, ensuring the implant's integrity is maintained without significant degradation over time, which is crucial for long-term applications. The structural integrity of phenolic compounds can be compromised by temperature, potentially altering their effects. Degradation would manifest as distinct peaks appearing at varying retention times. However, in our HPLC analysis, it was observed no degradation, as evidenced by consistent profiles before and after coating. When the mechanical properties of the NiTi alloy used in this study were examined, the micro-hardness value of the substrate material was measured in our previous studies and an average value of 425 Hv was obtained. In this study, the coating of this alloy with sea buckthorn extract, which has a high phenolic content, and the release properties of the extract were evaluated and its use as a drug delivery system was investigated.

According to the qualitative analysis of secondary metabolites in sea buckthorn (*Elaeagnus rhamnoides*) aqueous extract, it was seen that the extract has flavonoids and tannins as parallels with our previous study. Isorhamnetin glycosides as flavonoid and casuarinin as tannin were isolated from *n*-butanol extract [26]. Additionally, the total phenolic content of this extract was found as 137.25 μg gallic acid equivalent/mg extract (Fig. 1). The beneficial effects of these phenolic substances on osteogenesis have been well-documented, suggesting the implant's dual function as both a structural support and therapeutic agent in promoting bone health [31,32].

In the coating process, 18.1, 20.1, and 12.4 mg of extract were coated on approximately the same size (17x12 × 12 mm) of NiTi SMA material (samples 1, 2, and 3), respectively (Fig. 2). The surface morphologies of pre- and post-coated Ni–Ti alloy samples, as well as the elemental analysis of the pre- and post-coated Ni–Ti alloy, are presented in Fig. 3 a-d. Upon inspection of the pre- and post-coated samples (Fig. 3-a and 3-b), it was determined that no defects (such as precipitation or uncoated surface) were present that could alter the properties of the sample's morphology after coating. Additionally, the accumulation of extracts in the regions marked with arrows in Fig. 3-b was identified and when the EDS spectra were examined, the extracts coated in Fig. 3-d according to Fig. 3-c resulted in the formation of different spectra. In Fig. 3c, only peaks formed by Nickel and Titanium are present, indicating the composition of the substrate. Conversely, Fig. 3d reveals C, O, N, Na, Al, P, Cl, and Cu peaks most probably originating from the extract, alongside Ti and Ni peaks from the substrate, highlighting the successful incorporation of the extract into the coating.

In the analysis *in-vitro* extract release by HPLC, firstly it was seen that the extract profile was not changed by the coating process, it was the same as before and after (Fig. 4A and B). Two peaks were seen on chromatograms and the peak areas were evaluated for release of extract during five days. The most release was on the first day. During the other days, the release continued and lasted for 5 days. The next day there was about half the release of the previous day, and this is consistent for all three samples (Fig. 5). To analyze the release process of the extract, it was first calculated the release percentage for each sample at each time point. Then, it was plotted the average release (%) behavior over time and days for both the 1st and 2nd peak areas (Table 1, Fig. 5). Additionally, it was calculated the overall cumulative release percentage of the extract (Table 2). For the first peak, the highest release percentage, observed at 21.1 %, emerges during the initial hour of the first day. Conversely, the lowest percentage, a mere 0.7 %, manifests on the fifth day. Conversely, the second peak showcases its highest release percentage of 39.3 % also during the first hour of the first day. The lowest value for this peak, 2.3 %, appears on the third and fifth days.

Herbal extracts are prepared from herbal raw materials such as leaves, stems, roots, flowers, etc. by using different solvents, which have been used for medicinal purposes for centuries. Many plant extracts contain biologically active compounds that have been found to have therapeutic effects on various diseases. Several plant extracts have been found to have a role in bone formation and may be useful in the treatment and prevention of bone-related disorders. These extracts contain compounds that affect osteoblasts (bone-forming cells), osteoclasts (bone-resorbing cells), and the process of bone remodeling.

In this study, the Ni–Ti alloy coated with phenolic-rich extract was evaluated *in vitro* release properties by HPLC. Upon reviewing the literature, it has been observed that Ni–Ti alloy has been coated with various materials, such as tantalum, titanium nitride, calcium phosphate, graphene oxide/silver, etc. for biomedical applications [33–36]. However, the coating with plant extracts is introduced for

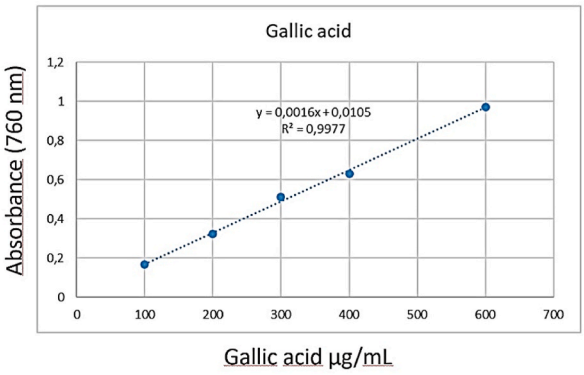


**Fig. 1.** Standard graphic of gallic acid

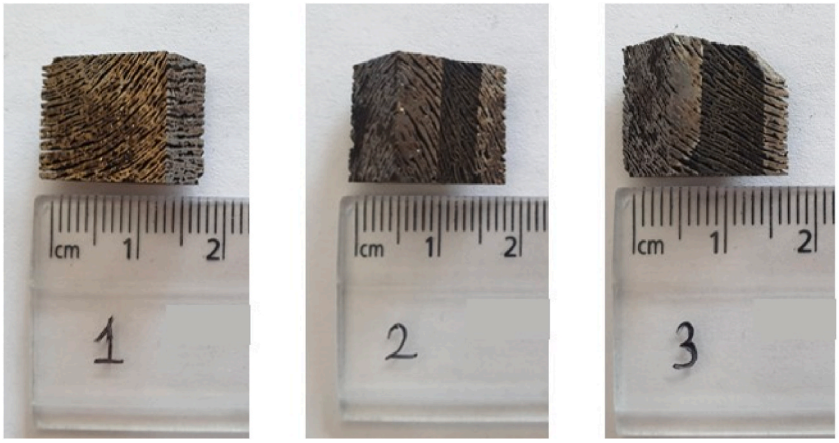


**Fig. 2.** Coated Ni–Ti alloy material

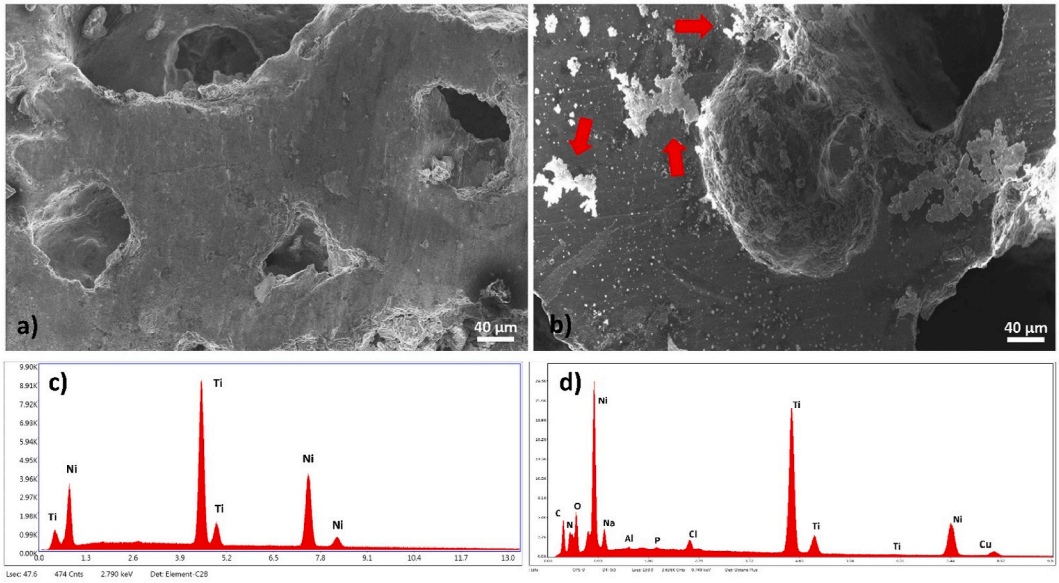


**Fig. 3.** SEM images of NiTi samples a) before coating b) after extract-coating c) EDS spectrum of the NiTi samples before coating d) EDS spectrum of the extract-coated sample

the first time in this study. This study opens up potential clinical applications for the Ni–Ti alloy coated with phenolic-rich extract, particularly in the treatment and prevention of bone-related disorders where sustained drug delivery is beneficial. However, challenges such as the scalability of the coating process, regulatory approvals, and patient-specific factors need to be addressed before these coated implants can be widely used in clinical settings.

Future research directions could include exploring different plant extracts, varying the coating thickness, and conducting *in vivo* studies to evaluate the performance of a living organism. Such studies would provide valuable insights into the long-term effects and biocompatibility of these coated implants, paving the way for their potential use in various biomedical applications. Moreover, investigating the environmental impact and sustainability of using natural extracts for biomedical applications could align with the broader goals of sustainable and green chemistry in the pharmaceutical industry, underscoring the importance of developing environmentally friendly and sustainable biomedical solutions.

## 4. Conclusion

In conclusion, our study focused on investigating the coating ability of Ni–Ti alloy using a hydrothermal method with sea buckthorn extract, emphasizing the mechanical integrity of the implant for biomedical applications. The extract contained phenolic compounds such as flavonoid and tannin. The analysis of phenolic compounds in the extract coating under physiological conditions revealed no

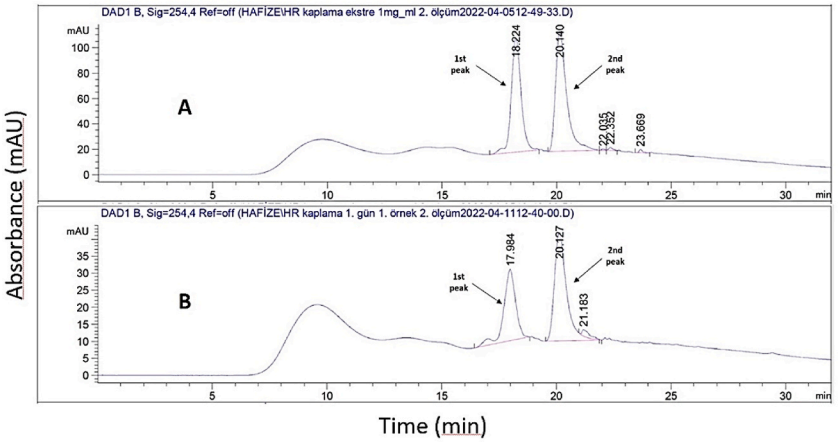


**Fig. 4.** HPLC chromatograms of extract (A) and releasing extract from material (B)

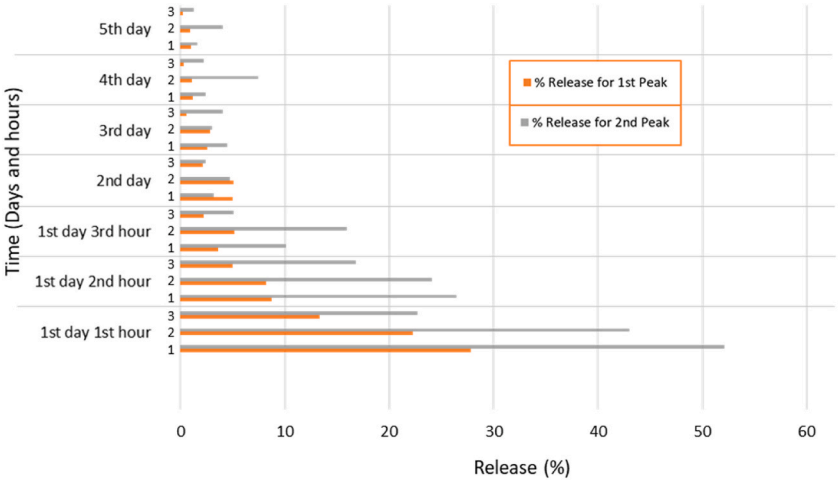


**Fig. 5.** Time-dependent % release graph of samples 1, 2, and 3

**Table 1**
Average release (%) for 1st and 2nd peaks.

| Hours and Days | 1st peak | 2nd peak |
|---|---|---|
| **1st day (1st hour)** | 21.1 | 39.3 |
| **1st day (2nd hour)** | 7.3 | 22.4 |
| **1st day (3rd hour)** | 3.7 | 10.4 |
| **2nd day** | 4.2 | 4.8 |
| **3rd day** | 0.7 | 2.3 |
| **4th day** | 0.9 | 3.5 |
| **5th day** | 0.7 | 2.3 |

degradation, ensuring the implant's integrity over time.

Successful coating of the Ni–Ti alloy with sea buckthorn extract was achieved, as evidenced by surface morphology evaluations and HPLC analysis. The incorporation of the extract into the coating was confirmed, indicating its potential as a drug delivery system. *In-vitro* release analysis by HPLC demonstrated sustained release properties over five days, with the highest release occurring on the first day.

This study introduces a novel approach of coating Ni–Ti alloy with phenolic-rich extract, offering potential clinical applications in the treatment and prevention of bone-related disorders. Further research directions include exploring different plant extracts, varying coating thicknesses, and conducting *in vivo* studies to assess long-term effects and biocompatibility.

**Table 2**
Cumulative release (%) for 1st and 2nd peaks.

| Hours and Days | 1st peak | 2nd peak |
|---|---|---|
| **1st day (1st hour)** | 27.8 | 52.1 |
| **1st day (2nd hour)** | 36.5 | 78.5 |
| **1st day (3rd hour)** | 40.3 | 91.2 |
| **2nd day** | 45.4 | 95.7 |
| **3rd day** | 46.6 | 99.7 |
| **4th day** | 48.3 | 100 |
| **5th day** | 100 | 100 |

## Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available.

## Ethics statement

Review and/or approval by an ethics committee was not needed for this study because there are no tests on humans or animals.

## CRediT authorship contribution statement

**Hafize Yuca:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Taha Çağrı Şenocak:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Oktay Yiğit:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Muhammet Gökhan Albayrak:** Writing – original draft, Methodology, Investigation, Data curation. **Zühal Güvenalp:** Writing – review & editing, Supervision, Resources, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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