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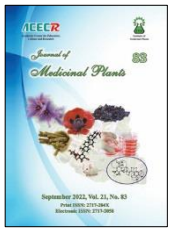

Research Article

Fabrication and optimization of physicochemical properties of nano-phytosome from *Punica granatum* L. peel enriched polyphenol extract

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ABSTRACT

Background: *Punica granatum* L. peel is a rich source of phenolic compounds such as punicalagin and ellagic acid that represent a major part of the fruit and many fruit by-products in Asian countries, mainly Iran. **Objective:** The purpose of this study is to design a value-added pomegranate peel product. **Methods:** For this purpose, a safe and environmentally friendly method of resin column chromatography was used to enrich the hydroalcoholic extract of pomegranate peel with punicalagin. The enrichment conditions were optimized in terms of column condition and the amount of extract used. The results were monitored and confirmed by HPLC-PDA. In order to improve the physicochemical properties of the enriched extract, the obtained extract was loaded onto nano-phytosome by thin-film hydration method. FT-IR confirmed the nano-phytosome structure. **Results:** The resin column was optimized at a 3:1 resin-to-extract ratio in an optimum contact time duration of 30 minutes (80.50 %) and a 2.63-fold increase in the phenolic compounds compared to the crude extract. The results indicated that encapsulation efficiency was equal to 58 %, the particle size was equal to 154.0 to 216.5 nm, and one-year stability was achieved with an optimal polydispersity index of 0.215-0.287 in terms of particle size. **Conclusion:** The enrichment of the crude extract before formulation and fabrication polyphenol enriched extract is a promising strategy to transform of pomegranate peel into a valuable product.

1. Introduction

Phenolic compounds, a plant secondary metabolite, provide significant pharmaceutical benefits for human health in addition to treat various diseases due to their antioxidant, anti-tumoral, antimicrobial and anti-inflammation properties [1, 2]. *Punica granatum* L., known as pomegranate, belongs to the Punicaceae family and is a fruit native to Afghanistan, China, Iran, and India, but it is cultivated throughout the Mediterranean region. Pomegranate fruits are widely used in producing juice. Despite some concerns about the peel's inedible part, it represents approximately 77 % of food

Abbreviations: EF-PGP, Enriched Fraction of *P. granatum* peels; FT-IR, Fourier-Transform Infrared Spectroscopy Analyses; DLS, Dynamic Light Scattering; ED, Effective Diameter (nm); Kcps, Average Count Rate; PDI, Polydispersity Index

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by-products in the mentioned region [3, 4]. Pomegranate peel is popularly used in traditional medicine. It is a rich source of polyphenolic compounds and has demonstrated significant pharmaceutical properties such as anti-infective, anti-oxidative, antimicrobial, anti-atherogenicity, hepato-protection, antidiarrheal, inflammation therapy, etc. [5, 6].

It is also known that the fractionation of plant extracts using polymeric adsorbent resin column chromatography facilitates the functionality of bioactive compounds based on their group characterization. Therefore, the extract enrichment enhances the phytoconstituent's bioactivity at a low dosage and removes undesirable compounds like sugar and chlorophylls, significantly increasing pharmaceutical's properties. However, technical information about optimizing resin amount, column condition, and the appropriate amount of crude extract are still not reported, neither preliminary nor comprehensive [7, 8].

Polyphenols have several limitations for use in differential formulation concerning stability, solubility, and bioavailability, which can be addressed to make these compounds more effective with longer shelf lives [9]. Lipid-based carriers with particular attention to the nano-phytosome should be a valuable strategy to improve the overall properties of phenolic compounds for food, cosmetic, and pharmaceutical properties [7, 10]. Although the encapsulation of pomegranate peels was widely investigated in Natural Polymer [11], the use of the lipid layer is still undiscovered and needs to be further investigated.

Therefore, this study focuses on two primary purposes; optimization of Diaion® HP-20, an adsorbent resin column chromatography, in the lab scale for the enrichment of *P. granatum* peels regarding phenolic compounds as a case study for the first time in detail. Formulation of enriched extract into nano-phytosome to improve phytoconstituents protection and stability experimentally evaluated in terms of particle size, product yield and dissolution assay (release control).

2. Materials and Methods

2.1. Chemical reagents

Lecithin with (CAS No. 8002-43-5) was purchased from Alfa Aesar, Thermofisher, Kandel, Germany. Sigma Aldrich provided the Punicalagin standard with a CAS No 65995-63-3. Ethanol (99 %) and dichloromethane were purchased from Scharlau (Barcelona, Spain). Formic acid, sodium phosphate monobasic and sodium phosphate dibasic heptahydrate was purchased from Merck (Darmstadt, Germany). The Diaion® HP-20, a polystyrene adsorption resin, was a gift sample from Resindion (Rom, Italia). HPLC grade solvents were purchased from Scharlau (Barcelona, Spain). EASY-pure II water purification system (Barnstead, Dubuque IA, USA) was used for obtaining ultrapure water.

2.2. Plant material and extraction procedure

P. granatum peels were collected from wild plants at the fruiting stage in Saveh district, Iran. The peels were dried at room temperature (25 °C) in shadow and kindly identified by Ali Sonboli at Medicinal Plants and Drugs Research Institute (MPDRI), Shahid Beheshti University, Tehran, Iran, with voucher number MPH-2345. The hydroethanolic extraction with ethanol/water ratio of 3:1 was carried out in five cycles, each cycle 2 L, 24 h, subjected to stirrer conditions at room temperature, as listed in Table 1. The extract was then filtered and concentrated by a rotary evaporator at 40 °C for further analysis (Fig. 1).

2.3. Enrichment optimization in terms of adsorption content and loading time

The concentrated extract was subjected to Diaion® HP-20 adsorbent resin to eliminate the unwanted structures (sugars and minerals) like aliphatic hydrocarbons, chlorophylls, sugar, etc., to provide an enriching phenolic fraction [7, 12]. The optimization procedures in terms of crude extract's content, Diaion®HP-20 volume, time of adsorbent, and color changes in tonalite are explained in Table 2. in detail.

Table 1. Extraction, enrichment and quantification of *P. granatum*

Variable	Dried peels (g)	Extraction		Enriched Extract	
		Extract (g)	Yield (%)	Enrich (g)	Yield (%)
P. granatum	445	217.88	48.96	96.72	32

*Yields were evaluated based on the total *P. granatum* peels dried powder, total extraction and the enrichment content in the powder.

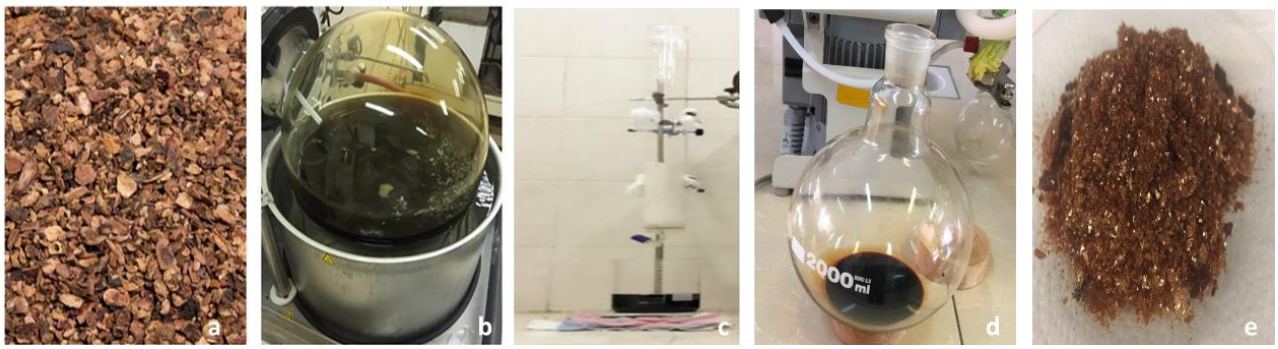

Fig. 1. Schematic of the extraction procedure from crude extract to enrichment; a) Punica grantum peels; b) the concentration of hydroethanolic extract; c) enrichment by Diaion® HP-20; d) the concentration of enriched fraction; and e) lyophilized powder

Table 2. Optimization of resin column regarding the efficient amount of crud extract and resin volume

No	Resin (ml)	Crude Extract (mg)	Time (min)	Color (tonality)	Adsorption Content (µl)	Adsorption Percentages (%)
1	2	2	0	Red	3835.38	38.35
2	2	4	10	Light Red	1517.18	15.17
3	2	6	20	Light Red	1446.84	14.46
4	4	8	30	Very Light Red	849.76	8.49
5	4	10	30	Very Light Red	403.84	4.03
6	4	12	40	Yellowish Red	185.90	1.85
7	4	12	40	Yellowish Red	166.54	1.66
8	4	12	40	Yellowish	149.87	1.49
9	4	12	50	Yellowish	25.96	0.25
10	4	12	60	Yellowish	23.57	0.23
11	4	12	720	Transparent	0.0	0.0
12	4	12	1440	Transparent	0.0	0.0

* Adsorption contents were evaluated considering punicalagin α, β content as standard.

The resin Diaion® HP-20 was activated by ethanol 99 % overnight and the column was neutralized by subsequent washing with ultrapure water. The process was started with 2 ml Diaion® HP-20 and 2 g hydroethanolic extract and continued to acquire an optimized volume of resin at 25 °C under an agitating condition. Twelve fractions were obtained and monitored by alternately changing colors and subsequently evaluated by HPLC-DAD. The adsorption content of each fraction, total adsorption percentage and optimized ratio between resin volume and crude extract were all monitored. The desorption of the enriching fraction was carried out by ethanol 99 %. Indeed, the total enrichment was done through resin column chromatography based on the content of the resin and crude extract obtained from optimization procedures. The total enriched fraction of *P. granatum* peels (EF-PGP) was first concentrated by rotary evaporator at 40°C, and subsequently frozen at -80 °C and lyophilized by freeze dryer (TOPT-10C, Xian Toption, China), and stored at 2-4 °C for further use.

2.4. HPLC characterization

HPLC-PDA-UV was used for the metabolite profiling of crude optimization fractions and EF-PGP following the previously developed methodology [11]. HPLC profiling was done at the analytical scale by Waters liquid chromatography apparatus consisting of a 2695 Separations Module (USA). An autosampler equipped with a 100 µl loop and Photodiode Array Detectors (PDA) using Waters (Ireland) Sunfire C_{18} column (150 mm, 3.5 mm × 3.5 µm). The solvent mixture was optimized as follow; MeCN + 0.1 % formic acid (A) and H_2O + 0.1 % formic acid (B), the following gradient was applied: 2 % (A) in 20 min; 2 % to 10 % (A) in 5 min; 10 % to 20 % (A) in 20 min; 20 % to 100 % (A) in 15 min; 100 % (A) in 10 min. The process takes 66 min with a flow rate of o.4 ml/min and an injection volume of 20 µl. The punicalagin calibration curve was drawn for the linearity range of 0.0005-0.1 mg/ml (y = 69897x + 4E + 06) for which correlation coefficient (R^2) was 0.99. The UV absorbance was measured at 254 nm.

2.5. Phytosome preparation

The nano-phytosome was prepared by the thin film hydration method taking into account the previously reported data with slight modifications [7, 8]. Lecithin and EF-PGP with an optimum molar ratio of (1:1) were used for nano-phytosome preparation. Some 100 mg of lecithin that was primarily dissolved in 3 ml of dichloromethane and vortexed for 5 min was added to the EF-PGP (100 mg) solution in ethanol. The final solution was refluxed at 50 °C, under stirred conditions for 2 h, and cooled down to 37 °C subsequently. The thin layer was formed using a rotary vacuum evaporator ranging from 60 to 200 rpm; 55 mbar vacuum pressure and dried by N_2 neutral gas for 1 min. The flask was sealed and left overnight in a desiccator. The hydration was undertaken by buffer (pH 5.5), at a vacuum pressure of 100 mbar, 100 rpm, and 40 °C, for 15 min. The nano-phytosome was subjected to ultra-sonication (probe sonication) for 20 min, at 70 % amplitude, in pulsation mood (5:1 s) to decrease and uniformize the particle size. The final solution was lyophilized by freeze-drying cycles to obtain the powder.

2.6. Fourier-transform infrared spectroscopy analyses (FT-IR)

The interaction between lecithin and EF-PGP in nano-phytosome had been previously

confirmed by Fourier transform infrared (FT-IR) apparatus (Frontier, PerkinElmer, Beaconsfield, UK) equipped with an attenuated total reflectance (ATR) accessory (PerkinElmer, Beaconsfield, UK), and operated by spectrum software (PerkinElmer, Beaconsfield, UK).

2.7. Particle size and zeta potential

The particle size distribution was evaluated by using Dynamic Light Scattering (DLS) equipment (Nanophox Sympatec GmbH, Claushtal, Germany). A small volume of the phytosome powder was dispersed in the ethanol and vortexed to ensure uniform distribution (350-450 Kcps). The particle size was measured in six runs of two minutes each. The Zeta potential (surface charge) was evaluated by NANO-flex II (surface charge reader), Colloid Metrix GmbH, Meerbusch, Germany, in six runs and the recorded duration was one minute.

2.8. Dissolution study

The dissolution assay (in vitro release study) was evaluated based on the release of punicalagin standard from particles over specific times and assessed in a gastrointestinal simulator containing 300 ml PBS buffer pH 2.1 under magnetic stirring condition (70 rpm) at 37 ± 2 ºC, according to the previous reports [8, 13]. A Spectra/Por® Dialysis membrane standard (RC Tubing, MWCO: 3.5 KD, width: 25 mm, diameter: 11 mm, USA & Canada). Some of the powder (20 mg) was dispersed in pure ethanol, placed into a dialysis membrane, sealed, compacted by the clamp and finally placed horizontally in the middle of PBS media. The punicalagin calibration curve (1.3 – 41.65 µg/L; y = 0.0517 + 0.0053, r^2= 0.99) was used to calculate the total amount of release (%). Letter “t” (time) corresponds to the presence of the characteristic peak of caffeine, assessed by UV-Vis spectrometry at a maximum wavelength of 254 nm (BioTek, Synergy HT, PMT 49984, USA). The punicalagin release was monitored in triplicate for almost 4 h at regular time intervals.

2.9. Statistical kinetic model

The mathematical kinetic models were used as a tool to obtain critical parameters that allow us to predict the release/dissolution mechanism, according to the previously reported data [13]. Therefore, punicalagin release from the phytosome carrier was evaluated through kinetic models. Zero-order (Eq. 1), First-Order (Eq. 2), and Korsmeyer-Peppas (Eq.3) were implemented to characterize a dissolution assay as described below:

$$F_t = F_0 + K_z t$$

Where "F_t" is the cumulative amount of the punicalagin released at the time "t", "F_0" is the initial amount of the punicalagin in buffer solution ($F_0 = 0$), "K_z" is the zero-order kinetic, and "t" is a time value.

$$F_t = F_{max} \times (1 - e^{K_f t})$$

Where "F_t" is the cumulative amount of the punicalagin released at the time "t", "F_{max}" is the maximum cumulative amount of punicalagin, and "K_f" is the first-order kinetic.

$$F_t / F_\infty = F_{KP}\, t^n$$

Where "F_t / F_∞" is the amount of punicalagin released until time "t", "F_{KP}" is the Korsmeyer-Peppas constant, "t" is a value of time, and n (release exponent; responsible for estimating different release mechanisms). Therefore, if $n < 0.43$, then punicalagin release follows pure diffusion (Fick law; Case-I transport). If $n > 0.43$, then punicalagin release follows a non-Fickian model; if $0.43 < n < 0.85$, then punicalagin release occurs based on the anomalous transport resulting in a combination

of Fickian diffusion and swelling release (Case II). If n = 0.85, release occurs based on the swelling and relaxation of the polymer matrix (Case-II). Finally, when n > 0.85, punicalagin release occurs based on the super case-II transport [14, 15].

3. Results

P. granatum peels are rich sources of phenolic compounds such as punicalagin α, β, and ellagic acid as main phytoconstituents [16]. Accordingly, punicalagin α, β was used as a standard for column optimization and *P. granatum* peels standardization. The resin adsorbent behavior had been monitored for 1440 minutes, taking into account the adsorption content of the punicalagin recorded by HPLC, as illustrated in Fig. 2. The best adsorption rate of 80.50 % was obtained in the first thirty minutes, and only a slight increase in adsorption was recorded at the end of 60 minutes (85.98 %). The optimization was concluded by 4 ml of resin Diaion® HP-20 which adsorbed 12 mg of crude extract (3:1 of resin to extract ratio) at an optimum duration of 30 minutes at ambient temperature. (Table 1). The Diaion® HP-20 column was used for scaling up by the information mentioned above. Some 217.88 g of the crude extract with 14.67 % of *α,β*-punicalagin was loaded onto the resin column; 96.72 g of EF-PGP with 38.75 % of *α,β*-punicalagin were obtained. The enrichment ratio and the yield of EF-PGP showed a 2.63-fold increase in the content of *α,β*-punicalagin as compared to the crude extract and 32 % procedure yield, respectively. Meanwhile, this study reported the preliminary condition of the resin column for the first time, associated with the correlation ratio between resin content and extract adsorption in the optimized time duration.

Conversely, the wise choice must consider carriers, a significant factor in improving the quality of the end product, with particular attention to the nano-phytosome due to its substantial similarity with the cell membrane. The efficient production yield was calculated at 58 %, excluding the amount of salt precipitated from the buffer solution, as depicted in Table 3 and Fig. 3. The synthesized nano-phytosome was proved the particle size between 154.0-216.5 nm during 365 days, with an optimum polydispersity index of 0.215-0.287 [17], as illustrated in Table 3, and Fig. 3. Indeed, the stability of lyophilized phytosome had been confirmed for almost one year. Nano-phytosme surface charge was confirmed in anionic mood and obtained -35.78 ± 1.95, -32.15 ± 7.47, and -32.15 ± 7.47 [11] corresponded to days 1, 180, and 365, respectively.

FT-IR spectra confirmed the interaction between lecithin and EF-PGP. A significant suppression in nano-phytosome as compared to the pure lecithin and EF-PGP might be a strong proof of effective interaction between ingredients. (Fig. 4), highlights the symptom of interaction between components. For instance, at a peak between 900-1200 cm^{-1}, the appearance of a mixture of lecithin and EF-PGP was confirmed. A significant suppression at a peak between 1500-1700 cm^{-1} appeared, which pointed to the overlap of peaks due to structure interaction.

The dissolution study was undertaken for nano-phytosomes in days 1, 180, and 365 to investigate their release profile in both fresh and after passing the storage condition. It confirmed the stabilization time of 170, 130, 130 min corresponds to nano-phytosome in days 1, 180, and 365, respectively (Table 4). A slight decrease in the stabilization time was observed from day one compared to days 180 and 360.

However, there was no significant indication of the nano-phytosome instabilities. All kinetic models showed that the best adjustment to nano-phytosome happens in day one, considering the R^2 factor. The release mechanisms followed the case I associated with valuing "n" in day 1. The stability of nano-phytosome and release mechanism were changed during times and transferred to anomalous release mechanism, a combination of Fickian diffusion and swelling release (case II) in days 180 and 360. The kinetic release adjustment (R^2) was decreased from day 1 to days 180 and 360, confirming the temporal changes in the structure of nano-phytosome. Therefore, the slight changes observed during various times should be associated with absorbing humidity in while in storage.

Moreover, the Zero-order kinetics showed the release follows Fickian ($R^2 \geq 0.800$) or non-Fickian. The zero kinetic models demonstrated that the Fickian mechanism occurred when punicalagin released at a constant and linear rate, independently of the concentration at any time [18, 11], Table 4, Fig. 5.

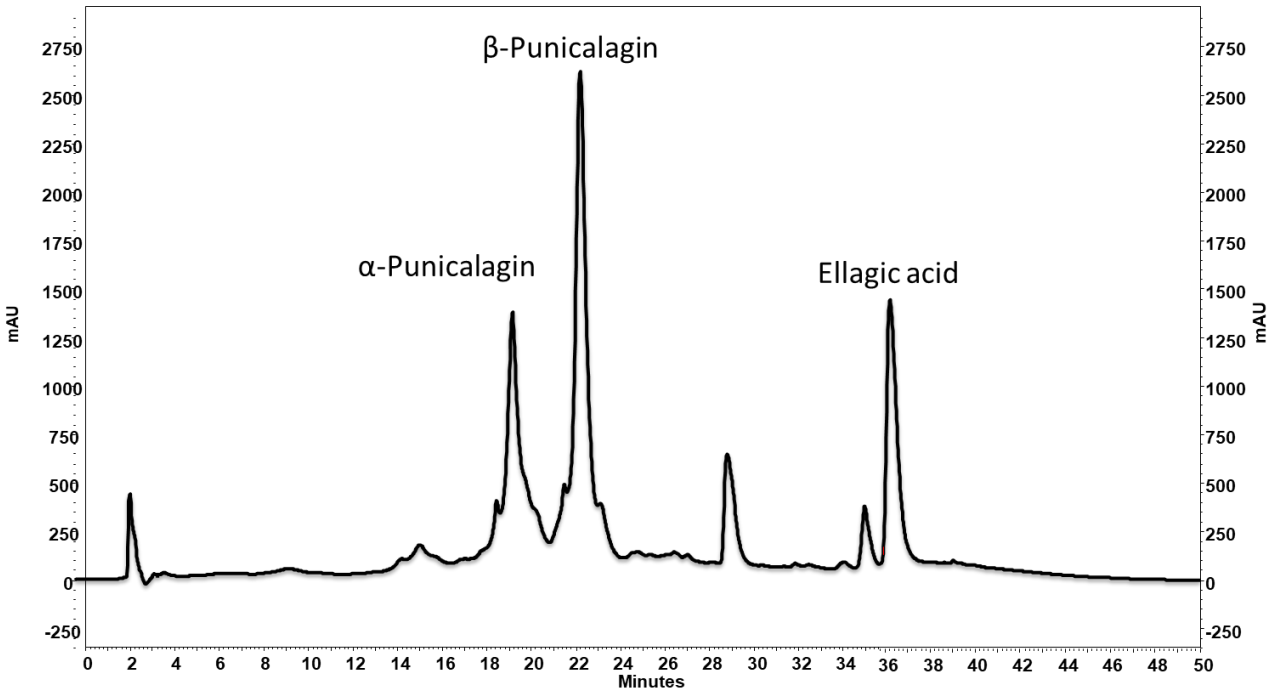

Fig. 2. HPLC chromatogram of P. *granatum* peels extract recorded at 254 nm

Table 3. The production yield and particle size stability for one year

Particle value	Yield (%)	Particle Stability								
		Day 1			Day 180			Day 365		
		ED	Kcps	PDI	ED	Kcps	PDI	ED	Kcps	PDI
Phytosome	58	154.0	453.8	0.267	173.1	458.1	0.215	216.5	437.2	0.287

* ED, effective diameter (nm); Kcps, average count rate; PDI, polydispersity index

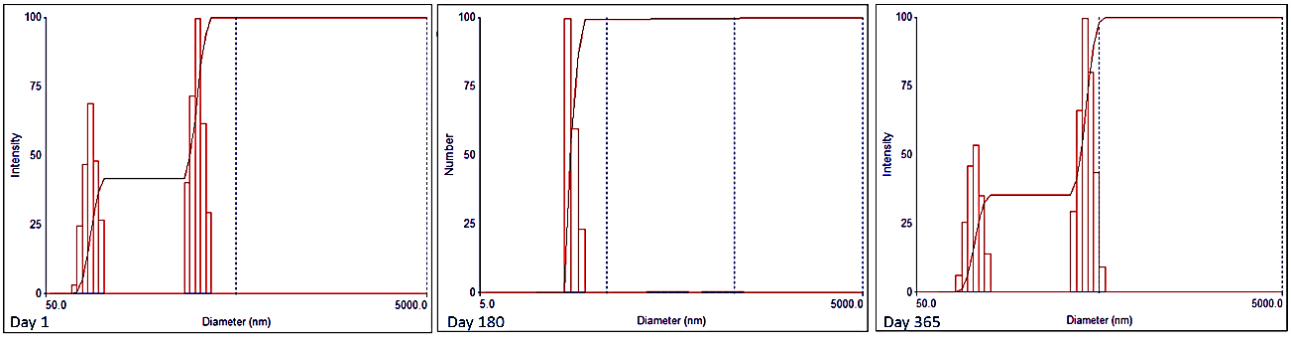

Fig. 3. Particle size distribution of nano-phytosome loaded by *P. granatum* peels

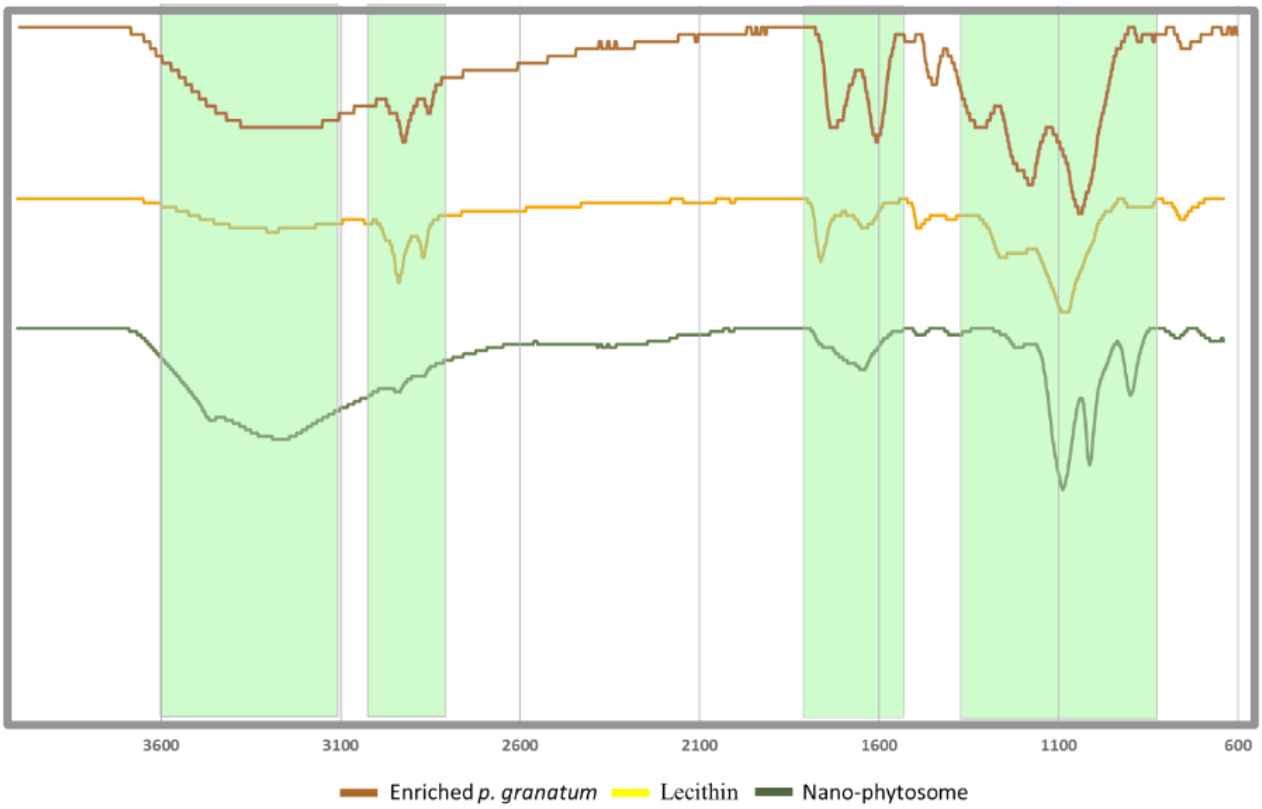

Fig. 4. FT-IR chromatogram of nano-phytosome lecithin and enriched fraction of *P. granatum* peels

Table 4. The control release mechanism of nano-phytosome during different times

Phytosome	ST	Zero-order			First-order			Korsmeyer-Peppas			
		F_0	K_0	R^2	F_1	K_{max}	R^2	K_{kP}	n	R^2	Mechanism
Day 1	170	0.002	0.04	0.98	0.0203	6.822	0.97	1.258	0.323	0.99	Case-I
Day 180	130	0.002	0.04	0.97	0.0002	261.26	0.97	0.465	0.664	0.66	Anomalous
Day 365	130	1.349	0.2	0.96	0.0085	5.970	0.86	0.283	0.527	0.86	Anomalous

ST, standardization time represented the specific time that shows the release turns to be constant. The criteria for determining the most appropriate model were based on the adjusted correlation coefficient in which $R^2_{Adj} \geq 0.800$ was considered an acceptable fit value and the best fitting model was the one with the R^2_{adj} closest to 1.

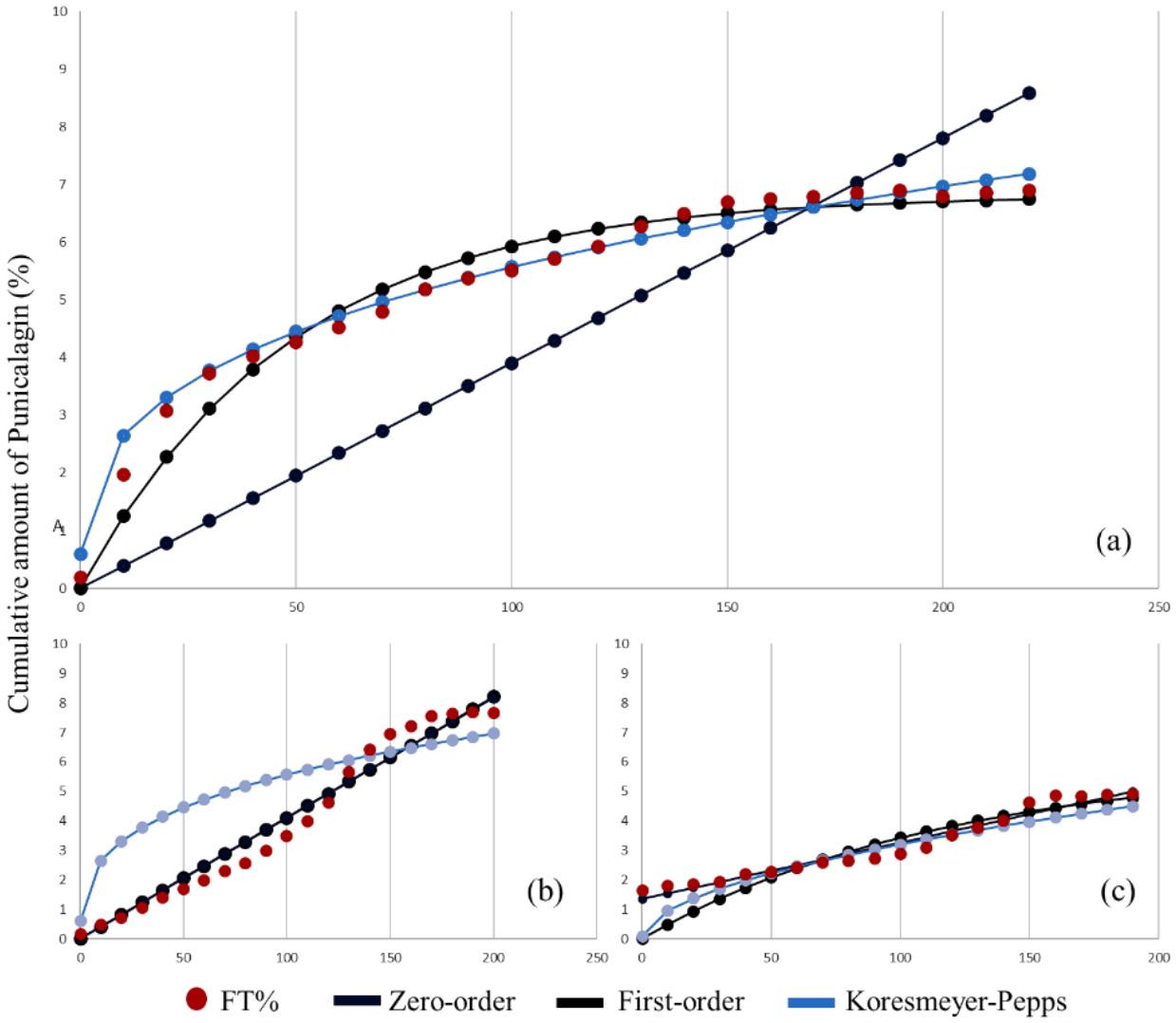

Fig. 5. Release mechanism nano-phytosome loaded by *P. granatum* peels during times. (a) day 1, (b) day 180, (c) day 365

4. Discussion

The use of adsorbent Diaion® HP-20 in the enrichment of phenolic compounds was confirmed previously [11, 12]. Considering that there was no comprehensive information about the column optimization condition and the ratio between the extract and resin Diaion, this experiment might open an opportunity for the researchers to significantly improve the quality of the final product by standardization of their initial extracts and signifying the quality of end product as well as saving times. Given that *P. granatum* peels are rich sources of phenolic compounds such as punicalagin α, β, and ellagic acid, it should be the best case study in current research not only due to environmental concerns (as a food by-product), but also because of the significant biological effect [16]. By the way, in terms of phytosome as a delivery carrier, our results were in agreement with the effect reported by Fathi *et al*, regarding particle size and stability of nano-phytosome, all in nanosize with a slight difference [8, 13], who reported the particle size of 70.8 nm in anionic charge in

terms of phytosome with four months stabilities while their used spray drying and secondary coating in their study [13]. Our results have confirmed one-year stability with no coating.

Despite considerable success, the interaction between an ingredient in the phytosome structure by FTIR led to lipid bilayer formation in phytosme formulae, as was previously reported by several researchers [7, 19, 20]. The suppression of critical peaks in the phytosome compared to the pure compounds indicates interactions between active ingredients and the lipid bilayer, as confirmed and proved previously [13]. Considering that prediction of release mechanism by the kinetic model was reported by different researchers [13, 15, 11], Korsmeyer–Peppas provided a better understanding of the release behavior, zero-order kinetic just confirmed a Fickian or non-Fickian, and first-order kinetic justified the super-slow release behavior [13, 15] which was clarified and in accordance to the experimental data.

5. Conclusions

The resin column optimization procedure was ended with 12 mg of crude extract (*P. granatum* peels), 4 ml of resin Diaion® HP-20 (3:1 of resin/extract ratio), and an optimum duration of 30 minutes (80.50 %). The EF-PGP reported 2.6 folded increase in phenolic compounds associated with punicalagin α,β as compared to the crude extract. Nano-phytosome was fabricated by the thin-film hydration method. The achieved product yield was 58 %, the particle size was detected at 154.0-216.5 nm, and stability was confirmed for almost 365 days with an optimum polydispersity index of 0.215-0.287 and favorable anionic surface charges. FT-IR confirmed the nano-phytosome structure. The dissolution assay recorded 130-170 min stabilization times and case I and anomalous release behavior associated with nano-phytosome days 1 to days 180 and 365.

Author contribution

D.K carried out the experiment and composed the manuscript; S.N.E supervised the project and reviewed the Draft; R.M.K contributed to the experimental part of the work and assisted in writing the draft of manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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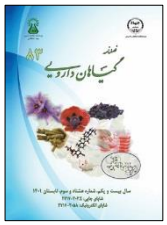

مقاله تحقیقاتی

ساخت و بهینه‌سازی خواص فیزیکوشیمیایی نانوفیتوزوم از عصاره پلی فنلی غنی شده پوست انار

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اطلاعات مقاله

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چکیده

مقدمه: پوست انار، منبع غنی از ترکیبات فنلی نظیر پونیکالاجین و الاژیک اسید است و بخش عمده‌ای از میوه انار و ضایعات کشاورزی را در کشورهای آسیایی و به ویژه ایران تشکیل می‌دهد. **هدف:** هدف از این مطالعه طراحی محصولی از پوست انار دارای ارزش افزوده می‌باشد. **روش بررسی:** برای این منظور، جهت غنی‌سازی عصاره هیدروالکلی پوست انار از پونیکالاجین، از روش ایمن و دوست‌دار محیط زیست کروماتوگرافی ستونی رزینی استفاده گردید. شرایط غنی‌سازی از نظر وضعیت ستون و مقدار عصاره مورد استفاده بهینه‌سازی شد. نتایج توسط HPLC-PDA پایش و تایید شد. به منظور بهبود خواص فیزیکوشیمیایی عصاره غنی شده، عصاره به دست آمده به روش هیدراتاسیون لایه نازک بر روی نانو فیتوزوم بارگذاری شد. ساختار نانوفیتوزوم توسط FT-IR تأیید شد. **نتایج:** ستون رزین با نسبت ۳ به ۱ رزین به عصاره به مدت زمان تماس ۳۰ دقیقه بهینه شد که منجر به بازده ۸۰/۵۰ درصدی و افزایش ۲/۶۳ برابری ترکیبات فنلی در مقایسه با عصاره خام شد. نتایج حاکی از راندمان کپسولاسیون برابر با ۵۸ درصد و اندازه ذرات برابر با ۱۵۴/۰ تا ۲۱۶/۵ نانومتر بود و پایداری یک‌ساله با شاخص چندپراکندگی بهینه ۰/۲۱۵ تا ۰/۲۸۷ از نظر اندازه ذرات حاصل گردید. **نتیجه‌گیری:** غنی‌سازی عصاره خام قبل از فرمولاسیون و ساخت عصاره غنی شده پلی‌فنلی یک استراتژی امیدوار کننده برای تبدیل پوست انار به یک محصول با ارزش می باشد.

مخففها: EF-PGP، فراکسیون غنی شده پوست انار؛ FT-IR، طیف سنجی مادون قرمز تبدیل فوریه؛ DLS، پراکندگی نور دینامیکی؛ ED، قطر موثر (نانومتر)؛ Kcps، میانگین نرخ شمارش؛ PDI، شاخص چند پراکندگی

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