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# Liposomes loaded with *Padina distromatic* alga extract: Physicochemical characterization and release behavior under simulated gastrointestinal conditions

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**ABSTRACT**: *In this paper, some nutraceutical properties of Padina algae extract were identified and its nanoliposomes were prepared to achieve a sustained release of bioactive substance. According to GC-MS analysis, butanoic acid was the foremost substance and n-Hexadecanoic acid was the second major chemical constituent in the extract. In addition, there are significant triterpenoids in the algal extract, which could be the possible reason for Acetylcholinesterase inhibitory activity, which has been recognized as a potential therapeutic strategy for the treatment of Alzheimer's disease. The formulation of algal extract-loaded nanoliposomes was optimized based on the variation of the wall-to-base ratio. The effect of lecithin in different ratios on the concentration of Padina alga extract mean size, polydispersity index, zeta potential, and encapsulation efficiency was evaluated. The results indicated that nanoliposomes loaded with algal extract showed a smaller size (318±3.05 to 59.95±0.19 nm), narrow size distribution, acceptable EE (15.7±0.92 to 52.8±0.52%), and good antioxidant activity (92 mg/ml). Morphological characteristics of the nanoliposomes using a transmission electron microscope reveal uniform particle with a spherical geometry. Moreover, the optimally formulated nanoliposomes show sustainable release of their contents into the simulated gastrointestinal fluids. These results can appear to the prospect of the possible use of this extract in the nano liposomal structure with a therapeutic approach.*

**KEYWORDS**: *Padina algae extract, nutraceutical properties, nanoliposome, release behavior*

## INTRODUCTION

Among marine organisms, algae are a rich source of biological compounds. In addition to vitamins, carotenoids, dietary fibers, proteins, and minerals, compounds such as terpenoids, flavonoids, sterols, polysaccharides, polyphenols, sargaquinic acid, sargacromenol and phophotin have been isolated from different types of algae [1]. These isolated compounds show various biological activities such as antioxidant activity, nerve protection, antimicrobial, liver protection, antiviral, etc. [2]. *Padina* is a brown alga belonging to the Dictyotaceae family that is widely found throughout tropical waters and usually along coral reefs. So far, about 80 species of the genus *Padina* have been identified in the world. The habitat of this algae has been reported in the tidal areas of 0-10 meters' depth of warm waters, due to the special shape, size, and color of its leaves, it can be easily identified from other algae. The 6 species of *Padina*, including *Padina* borersenii, have been identified on the northern shores of the Persian Gulf. These algae are one of the most abundant algae on the northern shores of the Oman Sea and the Persian Gulf [3, 4]. The studies conducted on the various properties of *Padina* algae extract show the presence of a high level of phenolic and antimicrobial compounds [5]. Also, these algae have a significant amount of polyphenols with antioxidant and anti-AChE (acetylcholinesterase) properties, which can be effective in the treatment of neurogenic disorders [6, 7].

The low delivery of phenolic compounds to the intestine due to instability in environmental conditions and food processes is considered one of the limiting factors for benefiting from the functional properties of these compounds [8]. On the other hand, these compounds are very important in food products to add functionality and improve nutritional and health benefits [9]. Therefore, before associating health interests with these compounds; The stability, absorption, distribution, and metabolism of each phenolic compound in the body are important points to consider.

The use of a method that protects these compounds from environmental conditions and ensures their controlled release at the desired time is very important and is considered a turning point in the production, trade, and industrial use of these compounds. Microencapsulation is one of the new processes in the food industry that allows the protection of useful compounds and their controlled release when necessary [10, 11, 12, 13, 14]. Therefore, today, the use of new methods to preserve and increase its bioavailability in the body has been the focus of researchers in this field. In the meantime, and based on the research conducted in recent years, the use of the liposome fabrication method for encapsulating bioactive compounds and improving their efficiency and bioavailability has attracted a lot of attention [15, 16]. A liposome is a microscopic vesicle consisting of two phospholipid layers, which surround a liquid space. The thickness of these lipid bilayers usually varies from 3 to 6 nm, and the size of liposomes formed from them can vary from 50 nm to 50 μm [17]. Due to the dual properties of their constituents, liposomes offer the possibility of delivering hydrophilic and lipophilic bioactive compounds. Properties such as low intrinsic toxicity, biodegradability, and non-immunogenicity have made liposomes very suitable carriers for new delivery systems of bioactive compounds. Characteristics such as low intrinsic toxicity, biodegradability, and lack of immunogenicity, have made liposomes a very suitable carrier in new delivery systems of bioactive compounds [18].

In recent years, the application of liposome structures for the encapsulation of phenolic compounds has been successfully used as an effective carrier for delivering functional ingredients in food systems [19, 20, 21, 22]. In 2017, Pinilla et al. studied the physical properties of liposome-loaded garlic extract. The results showed that liposomes are a suitable system for collecting the volatile active compounds of garlic. Later on, phenolic

compounds of pistachio green hull extract encapsulated in liposomes prepared by the thin hydration method showed good encapsulation efficiency, taking into account the risks of using chemicals for liposome preparation and limiting the use of this method in food applications [20]. In another study, nanoliposome formulation with algal extract was optimized based on response surface design. The results showed that the synthesized nanoliposomes had good stability under storage conditions and could control the release of phenolic compounds at different pH [7]. However, there is limited information on the release behavior of these structures in simulated gastrointestinal conditions.

This study was conducted to consider the functional properties of the bioactive compounds of *Padina* extract for possible use in a therapeutic approach. In addition, to increase the bioavailability of the extract, encapsulation was carried out in the nanoliposome structure. Accordingly, the study was focused on determining the optimal conditions for encapsulating algae extract in nanoliposomes. The feasibility of the encapsulation process was achieved using physical studies including particle size, zeta potential, and transmission electron microscopy along with the study of molecular interactions using Fourier-transform infrared spectroscopy (FTIR). On the other hand, the release behavior of the nanoliposomes in the gastrointestinal tract was investigated to evaluate the ability to deliver bioactive compounds to the target point.

## EXPERIMENTAL SECTION

***Materials:***

Phosphatidylcholine was prepared by Acros Organics, Belgium, and other chemicals in the analytical grade was supplied by Merck & Co. *Padina* brown algae was collected from the Persian Gulf coast in Sistan and Baluchestan Province (Chabahar port).

***Preparation of algal extract:***

Extraction was done using the ultrasonic method. In this method, indirect ultrasonic waves with a frequency of 70 kHz at a temperature of 25°C were used for one hour. Briefly, sea salt is removed from algae by washing with distilled water. After drying, the algae were milled and 10 grams of powder were placed in the beaker containing 100 ml solvents in equal ratios of methanol, n-hexane, and n-butanol. The mixture is placed in an ultrasonic bath for 1 hour. Filtered using Whatman No. 1, dried in a low-pressure rotary evaporator, and stored in containers at 4°C before use [23].

***Chemical composition***

The chemical components of *Padina* extracts were identified by gas chromatography-mass spectrometry, GC-MS (MSD, 5977B, detector, Agilent Technology, USA). For this purpose, 0.2 µL of each extract was introduced into a DB-5 chromatography column with the following specifications: injection temperature 200°C; Temperature 50-250°C (10°C per minute). This flow is 1 ml/min as carrier gas. The ionization voltage is 70 volts. Retention time and mass spectra of products were compared to those of the National Library of Standards and Technology for component identification.

***Total phenolic content:***

Total phenolic content was determined by the Folin-Cicocalto method. 100 µL of this preparation is mixed with 2 ml of 2% sodium carbonate, and after keeping it at room temperature for 2 min, 100 µL of Folin-Cicoccalto reagent (1-1 dilution in deionized water) is added. The prepared sample is placed in a shaker to completely

combine the reagent in the solution, and then it is placed at room temperature for 30 minutes to complete the reaction. Samples were absorbed in 720 nm using a spectrophotometer (Pharmacia Biotech, Novaspec II, LKB, England). Phenolic concentrations are expressed as mg gallic acid equivalents per gram of dry matter (GAE/gdw) [24].

***Total Antioxidant Activity (TAA)***

The DPPH radical capacity method was used to determine the antioxidant activity of P. distromatica extract. 1 ml of the extract was mixed with 1 ml of 2,2-diphenyl-1-picrylhydrazyl solution (0.1 mM) for 30 minutes. Absorbance was then measured in the dark with a UV spectrophotometer (Pharmacia Biotech, Novaspec II, LKB, England) at a wavelength of 517 nm. Ascorbic acid and butylated hydroxyanisole (BHA) were also measured as standard samples. The percentage of free radical inhibition (inhibitory activity) was calculated from this equation.

$$\text{Percentage of free radical inhibition } (\%) = (\text{Abs blank} - \left(\text{Abs}\frac{\text{sample}}{\text{blank}}\right) \times 100 \quad (1)$$

By using the curve of free radical inhibition percentage against extract concentration, $IC_{50}$ (the extract concentration with 50 percent inhibition) was estimated as the number of antioxidants needed to reduce the DPPH concentration by 50%. A lower $IC_{50}$ equals the higher antioxidant activity of the algal extracts [25].

***Acetylcholinesterase inhibitory activity***

Cholinesterase inhibitory activity was measured according to the method of Elman et al. [26]. Briefly, 125 µl of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid (DTNB), 25 µl of 0.075 M acetylthiocholine iodide (ATCI), and 50 µl of 0.1 M phosphate buffer (pH 8.0 µl) were mixed. U/ml acetylcholinesterase (AChE) and absorbance were measured at 412 nm using a spectrophotometer (Pharmacia Biotech, Novaspec II, LKB, England) every 20 seconds for 2 minutes. Additionally, the dispersion was added to a 96-well plate, then 25 µL of each algae extract at different concentrations was added, and then the absorbance was measured at 405 nm. Percentage of cholinesterase inhibition:

$$\%\ \text{Anti} - \text{AChE activity} = [(\text{E} - \text{S})/\text{E}] \times 100 \quad (2)$$

Where E is the enzyme activity in the absence of algal extracts and S stands for the enzyme activity in the presence of algal extracts

***Preparation method of nanoliposomes containing Padina extract*:**

Liposomes containing *Padina* algae extract were produced using the heating method. At first, the *Padina* algae extract solution was prepared in 2 concentrations (0.7% and 2%) with deionized water. Then, phosphatidylcholine (2.5% and 4.5%) was added to the solution containing the extracts. In the end, glycerol at a concentration of 3% was added to the prepared solutions. The prepared samples were placed on a hot plate for 1 hour at a temperature of 70 degrees Celsius and a stirring speed of 1000 rpm. After the mentioned time, Liposome samples were incubated for 1 h at room temperature to increase their stability, and after this time, they were transferred to the refrigerator at 4 degrees Celsius [27].

***Measurement of particle size and distribution:***

The size distribution and diameter of the particles were measured using a particle size analysis (Cordouan Technologies device, France) based on the laser diffraction method which is about the relationship between particle size and the angle and intensity of scattered light [28].

***Measurement of zeta potential of particles:***

The Zeta potential of liposome particles prepared using Zetasizer (Colloids & Interface Instruments Zeta Compact, France) as described by Bouarab et al. [28].

***Determining the encapsulation efficiency of the extract:***

The encapsulation efficiency of nanoliposomes was determined using the Folin-Cicocalto method. Nanoliposomes were separated using a centrifuge made by Helmer, Germany, model 236HK (36000 g, 30 minutes). Then the content of total phenol in the supernatant (the amount of free extract) was measured and the encapsulation efficiency of the extract was determined based on the following Equation [29].

$$EE\% = (\mathrm{Pi} - \mathrm{PS})/\mathrm{Pi}) \times 100 \quad (3)$$

where Pi is the total amount of phenolic compounds and Ps represents free phenolic compounds in the supernatant.

***Scanning transmission electron microscope***

A 20 µl aliquot of the liposome suspension was placed on a copper-coated grid for 2 min and then stained with 20 µl uranyl acetate for 1–2 min. After drying at room temperature, the morphology of the samples was examined using a transmission electron microscope . (Omega, Leo912AB, Germany) at a voltage of 100 kW.

***FTIR analysis***

FTIR observations of loaded and empty nanoliposomes as well as *padina* extracts were performed using a Thermo FTIR spectrometer (AVATAR 370 FT-IR (USA)) in wavenumbers of 4,000 to 400 $cm^{-1}$.

***Release behavior of nanoliposomes***

The release behavior of phenolic compounds from nanoliposomes was investigated using the procedure described by Basiri et al. [30]. Briefly, with some modifications, nanoliposomes with phenolic compounds were separated from a nanoliposome device placed in a dialysis bag by centrifugation and transferred to a container with 100 ml of simulated gastric fluid (HCl solution 0 .1 N, pH = 1.2). The discharge pattern was examined at a predetermined period of 2 hours. The dialysis bag was then removed and transferred to a container containing 100 cc of simulated intestinal fluid (SIF, sodium-potassium dihydrogen phosphate buffer solution adjusted to pH 7.4). The extracted phenolic compounds were recorded at regular intervals of 6 hours. The experiment was performed at 37°C for 50 min under continuous shaking. Phenolic compounds were quantified using the Folin-Ciocalteu method and cumulative release was calculated using the following equation. The measurements were repeated three times.

$$CR\ (\%) = \sum_{t=0}^{t} \frac{P_t}{P_0} \times 100 \quad (4)$$

***Statistical analysis***

To analyze the data, a factorial experiment with a completely random design was used. Duncan's multiple test will be used to determine the difference between means at the 95% confidence level and Minitab software version 17 will be used for statistical analysis.

## RESULTS AND DISCUSSION

***Chemical composition***

As shown in Table 1, the wide array of nutraceutical appreciable phytochemical components belonging to different chemical groups including phenols, alcohols, esters, fatty acids, alkenes, and ketones were observed in GC/MS

analyses. Names of these phytochemical substances, their retention time (RT), and their area are listed in Table 1. According to GC-MS analysis, 1-Butanol-2-methyl-(S), 4- Heptanol, 4- Heptanone,3-methyl, Butanoic acid, butyl ester, Palmitoleic acid, Butane, 1,1-butoxy, n-Hexadecanoic acid, Cis-Vaccenic acid, and Stigmasta-5,24(28)-dien-3-ol(3β,24Z) were identified. Among the components, butanoic acid was the foremost substance and n-Hexadecanoic acid was the second major chemical constituent in the extract. Rosado-Espinosa et al. [31] also reported that hexanoic acid 2-ethyl was identified in sargassum buxifolium algae. It is worth noting that several substances have been identified in marine algal extracts with antioxidative, antiviral, and inhibitory activities. Moreover, Polyunsaturated fatty acids (PUFAs)were introduced as an antimicrobial, antiviral, and anti-inflammatory agent [32], which were found in the extracts in the current investigation.

***Table 1. Major phytochemical compounds identified by GC-MS analysis in extracts of P. distromatic***

| Phytochemical compound | RT (min) | Area sum (%) | molecular weight (g/mol) | Molecular Formula |
|---|---|---|---|---|
| 1-Butanol-2-methyl-(S)- | 6.219 | 1.18 | 88.148 | $C_5H_{12}O$ |
| 4- Heptanol | 8.382 | 5.99 | 116.2 | $C_7H_{16}O$ |
| 4Heptanone,3methyl | 9.091 | 3.56 | 128.22 | $C_8H_{16}O$ |
| butanoic acid, Butyl ester | 10.831 | 28.25 | 144.21 | $C_8H_{16}O_2$ |
| 3-Deoxy-d-mannitol | 11.666 | 5.04 | 166.15 | $C_6H_{14}O_5$ |
| Silane,dimethylisobutoxybutoxy | 13.772 | 2.59 | 188.38 | $C_{10}H_{24}OSi$ |
| Butane,1,1-dibutoxy | 17.994 | 7.32 | 202.33 | $C_{12}H_{26}O_2$ |
| Tetradecanoic acid | 30.074 | 3.78 | 228.37 | $C_{14}H_{28}O_2$ |
| Hexadecanoic acid, methyl ester | 32.191 | 2.02 | 270.45 | C17H34O |
| Palmitoleic acid | 32.465 | 5 | 256.4 | $C_{16}H_{32}O_2$ |
| Cis-Vaccenic acid | 34.594 | 8.02 | 282.461 | $C_{18}H_{34}O_2$ |
| stigmasta 5,24(28)-dien-3ol(3β,24Z)- | 47.46 | 4.27 | 412.7 | $C_{29}H_{48}O$ |

***Acetylcholinesterase inhibitory activity***

To remedy Alzheimer's disease, various potential therapeutic strategies have been conducted. Among all therapeutic approaches, AChE inhibitors have been considered one of the most effective ways to improve cholinergic neurotransmission [33]. The AChE inhibitory activity of the extract is shown at 42.50%. Earlier, as observed in GC-MS chromatograms, there were significant triterpenoids in algal extracts. Evidence on the relationship between the presence of triterpenoid compounds and AChE inhibitory activity has been reported in the literature. Syad et al. [33] evaluate the anti-Alzheimer potential of Sargassum wightii through the evaluation of AChE inhibitory activities. The findings of these researchers indicate that the existence of triterpenoids in the algal extracts could be a possible reason for AChE inhibitory activity.

***Total antioxidant activity (TAA)***

The antioxidant activity of the *Padina* algal extract was presented in terms of IC50 and was calculated as 92 mg/ml. The IC50 of a constituent has an indirect correlation with its antioxidant activity, meaning that the higher antioxidant activity of an extract corresponds to a lower IC50 [25]. Savaghebi et al.[8] reported antioxidant activity of the Sargassum boveanum algae extract based fractionated by three solvents (dichloromethane, ethyl acetate, and n-butanol). The results showed that the ethyl acetate fraction, compared to other solvents, had the highest antioxidant activity (171.4 mg/ml), which has lower antioxidant activity compared to the *Padina* algae extract reported in this study.

***Nanoliposomes characterization***

***Particles size***

The effect of changing the ratio of wall to core material on the size of liposome particles is shown in Table 2. The average particle size was in the range of 59-318 nm. The largest particle size (318 nm) was observed for the sample with the highest level of lecithin (4.5%) and algae extract (2%) in the formulation. On the other hand, the lowest particle size (59 nm) was observed in the sample containing the lowest level of wall material (2.5% w/w) and the highest level of the algae extract (2%). In this sample, the ratio of the wall material to the core was at a relative level of almost equality. Similar results have been reported for other liposome systems [8, 34]. These results indicate that there is an optimal level of wall-to-core ratio in nanoliposome systems. The dependence of particle size on the core-to-wall ratio can probably be expressed as an increase or decrease in the number of particles with size variation [35].

The polydispersity index is one of the important parameters in evaluating the structural properties of nanoliposomes [36]. As can be seen in Table 2, the polydispersity index as a measure of uniformity of particle size distribution was in the range of 0.15-0.35, which is dependent on the ratio between the lecithin to extract. The obtained values of this parameter indicate the uniform distribution of the formed particles

*Table 2. Particle size, PDI, zeta potential, and EE results of the prepared liposomes.*

| Lecithin to *Padina* extract ratio (w/w) | Particle size (nm) | PDI | Zeta (mv) | EE (%) |
|---|---|---|---|---|
| **0.7-2.5%** | 149.64±2.47$^{b}$ | 0.34±0.017$^{a}$ | -37.59±1.23$^{b}$ | 24.3±1.04$^{c}$ |
| **0.7-4.5%** | 78.56±1.57$^{c}$ | 0.35±0.021$^{a}$ | -40.35±0.95$^{a}$ | 15.7±0.92$^{d}$ |
| **2-2.5%** | 59.95±0.19$^{d}$ | 0.31±0.032$^{a}$ | -34.96±0.89$^{c}$ | 52.8±0.52$^{a}$ |
| **2-4.5%** | 318±3.05$^{a}$ | 0.15±0.016$^{b}$ | -33.8±1.03$^{d}$ | 38.2±0.86$^{b}$ |

Different letters represent a significant difference from one another ($p< 0.05$).

***Zeta potential***

The zeta potential parameter is an indicator of the physical stability of nanoliposomes in suspensions. The large surface charge indicates high repulsion between particles and as a result the unwillingness to form aggregate, hence implying a high level of stability. This parameter is a function of the surface charge of lipid vesicles, surface adsorbed layers, and environmental conditions. In addition to the importance of this parameter in structural stability, in in vivo conditions, the surface charge of nanoliposomes affects the circulation time of particles in the blood [37].

Table 2 shows the magnitude of the zeta potential of nanoliposome particles containing *Padina* algae extracts under the affected of variation the wall-to-core ratio. As the results show, this parameter varies between -33.8 to -40.35 mV for the fabricated nanoliposomes. Zeta potential less than -10 mV and more ideally less than -30 mV, by creating a high repulsion between the colloidal particles in the system, creates favorable stability in the suspensions containing nanoparticles [38]. Therefore, the results obtained in this research are proof of the stability of nano liposomal systems. The highest magnitude of zeta potential (-40.35 mV) for nanoliposome containing 4.5% of wall material and 0.7% of *Padina* algae extract and the lowest value (-33.8 mV) for liposome containing 4.5% of wall material and 2% of the extract was obtained. The change in the magnitude of the zeta potential under the influence of the level of the extract and wall material can indicate the interaction between the wall composition and the phenolic components present in *Padina* algae extract [39]. The phenolic compounds present in the encapsulation medium, in addition to being trapped in the internal structure of liposomes, are also absorbed on the surface of the vesicles. Hence, some phenolic ingredients in the seaweed extract can bind to the negative phospholipid groups on the surface of the nanolipid membrane [40]. In addition, relatively positively charged compounds in the extract around the surface of nanoliposome particles cause a higher surface charge of the formed vesicles [36]. Savaghebi et al. [8] reported that the zeta potential of Sargassum Boveanum algae extracts encapsulated in nanoliposomes differed from −37.3 to −50.7 mV. Pinilla et al. [20] reported similar results in zeta potential in nanoliposomes loaded by garlic extract varied between -24.3 to -16.2. Machado et al. [40] observed lower zeta potential values in liposomes containing phenolic compounds of Spirulina LEB-18 extract (-11 mV) than in non-loaded liposomes (-46.7 mV).

***Efficiency of encapsulation***

As the results show in Table 2, the encapsulation efficiency improved by increasing the concentration of the extract at a constant level of lecithin. Based on the results, the highest level of encapsulation (52.8 ± 0.3%) of *Padina* algae extract was obtained at a concentration of 2% of the extract and 2.5% of lecithin. Increasing the lecithin level by affecting the viscosity of the system reduces the free movement of phenolic compounds. Hence, the encapsulation efficiency decreases and a lower level of phenolic compounds is trapped inside the structure of lipid vesicles [17]. Savaghebi et al (2020 reported that the highest efficiency of phenolic inclusion in nanoliposomes reached approximately 50% [8]. Bagnosat et al. (2016) have similar reports for the encapsulation of Spirulinas extract (55%) in Liposomes [41]. In addition, an entrapment efficiency of 47.5% was obtained for encapsulating garlic extract in liposomes [20], which is consistent with our results.

***Transmission electron microscope***

The structural characteristics of nanoliposomes were investigated using transmission electron microscopy. As can be seen in Figure 1, the formed nanoliposomes have a spherical structural morphology. The size of the particles as well as polydispersity is consistent with the results of the laser light scattering method. Spherical morphology, uniform particle size, and reduction of particle size in the nanometer scale, in addition to improving the delivery of the loaded material, make the nano liposomal suspension stable during storage. In general, several factors such as the ratio of phospholipid to the loaded active substance, the method of nanoliposome production, temperature, and production time are effective on the size and structural morphology of the formed particles [42].

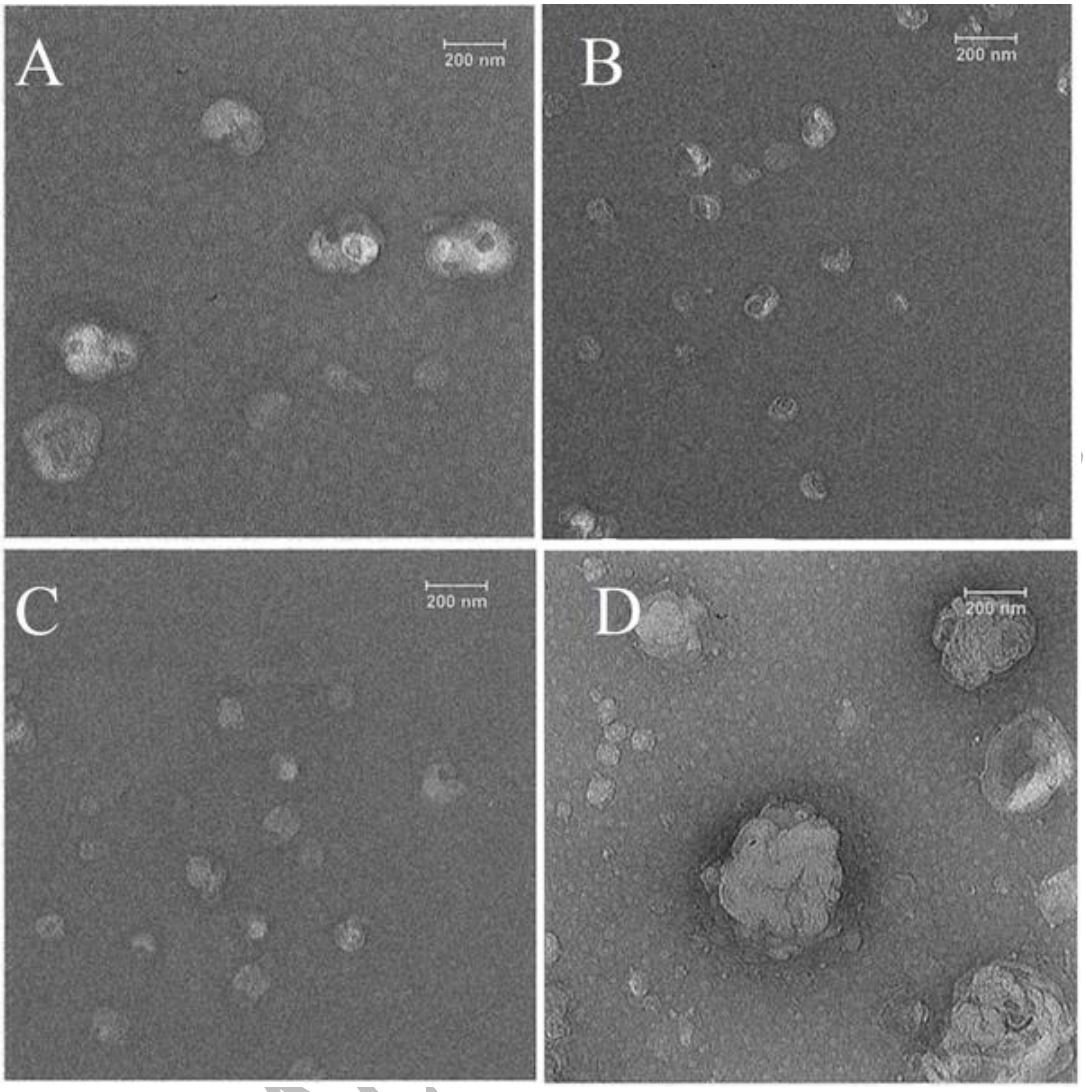


***Figure 1- TEM images of liposomes as the different concentrations of lecithin and Padina extract in 10000x magnitude. A) 0.7-2.5% lecithin- Padina extract (w/w), B) 0.7-4.5% lecithin- Padina extract (w/w), C) 2-2.5% lecithin- Padina extract (w/w), D) 2-4.5% lecithin- Padina extract (w/w)***

***FTIR spectra analysis***

FTIR ***spectra*** were applied to confirm the capsulation of algal extracts in nanoliposomes. Figure 2 shows the FTIR pattern of liposomes in loaded and empty as well as free alga extract. The characteristic peaks of phospholipids in the non-loaded nanoliposome sample were found at wavenumber 3384 (OH bond lecithin and water), 2927, 2858, and 1738 correspond to $CH_2$ asymmetric stretching vibration. in addition, wave numbers 1043 and 1229 respectively showed the peaks corresponded to the symmetric and asymmetric stretching of phosphate groups (PO2), [43].

Broad peaks located in 3300 and 3400 /cm corresponded to C-H, OH, and N-H stretching observed in the FTIR spectrum of algal extract which related to amide hydroxyl and amine groups of main phenolic compounds [44, 45].

With loaded extract into the nanoliposomes, the wavelength of some absorption groups changed to higher or lower values, indicating that phenolic chemicals interact with the nanoliposome. In contrast to algae extraction and empty nanoliposome samples, the O-H bond was sharpened and dislocated to a wavelength with a higher value

(3411), as shown in Figure 2. According to Tang et al. [46], this change may be the result of hydrogen bonding between the hydroxyl group of phenolic chemicals in algal extracts and polarization of phospholipids. After loading, the peak phenolic compounds (symmetric vibrations and asymmetric stretching of the CH2 group) at 2858 cm and 2927 cm were sharpened and changed to 2859 cm and 2929 cm in empty nanoliposomes. These changes indicate that some phenolic chemicals are present in the bilayer membrane of nanoliposomes [47].

Furthermore, after encapsulation, the peak associated with C=O stretching in the algal extract spectrum shifted from wavelength 1654 to 1642 which indicated an interaction between phenolic compounds in an extract with lecithin with forming hydrogen bonds [21]. Based on these results, phenolic chemicals were successfully incorporated into liposomal structures.

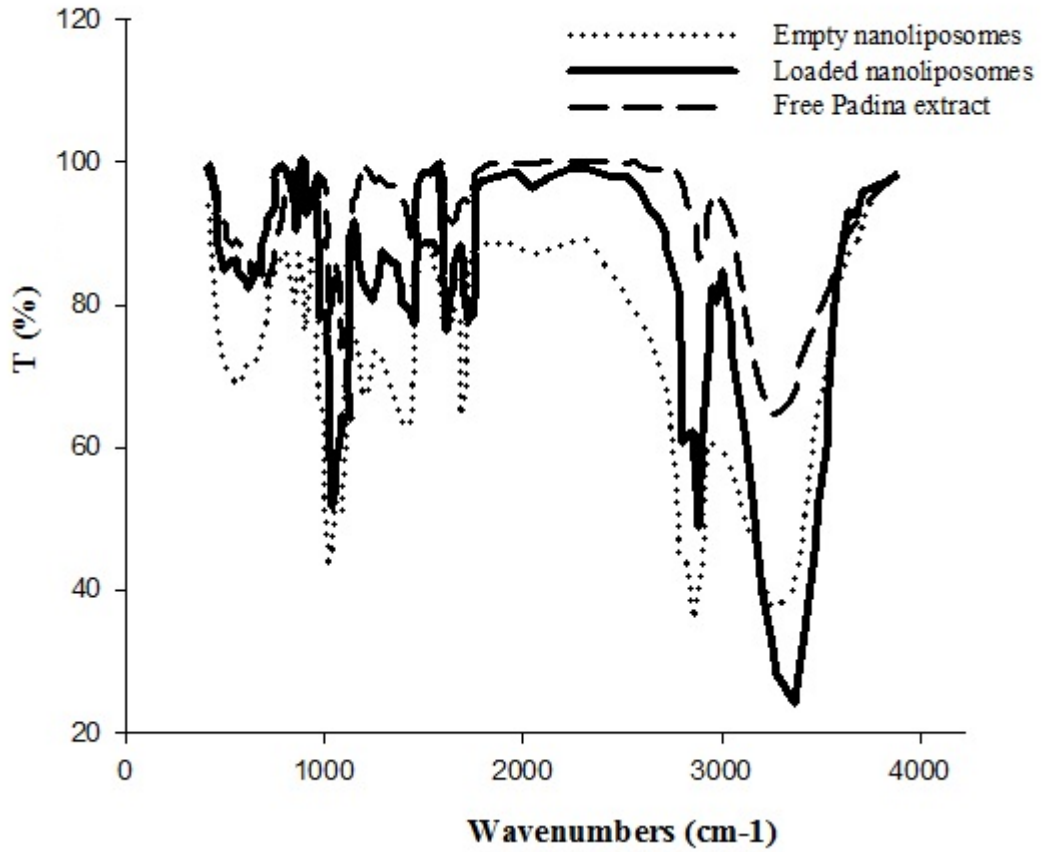


***Figure 2- FTIR spectrum of free alga extract and loaded and empty nanoliposomes***

***Release behavior of nanoliposome***

The activity of encapsulated bioactive compounds depends on their intended delivery. Among the various carriers used to deliver bioactive compounds, nanoliposomes are unique delivery vehicles that play multiple roles in improving the efficacy of functional components. The significant role of these carriers is in the targeted and controlled delivery of loaded compounds to achieve the maximum benefit of their bioactive properties. therefore, the most efficient nanoliposome system was selected according to the experimental data in the above sections to investigate release behavior in simulated gastric fluid for 2 h, and simulated intestinal fluid for 6 h. The release behavior of nanoliposomes is shown in Figure 3. The results show that 13.56% of phenolic compounds are

released in SGF in 2 hours and its release reaches 40.6% in SIF after 8 hours. The cumulative release pattern of compounds loaded in nanoliposomes was less than 50%. These results indicate that the nanoliposome system follows a sustained release pattern with an Initial burst and reaching a relatively constant rate of release. This pattern can correspond to the entrapment of phenolic material along the external and inner layers of the nanoliposome which can be released more quickly from the surface and with a delay from the inner layer [47,48]. Liu et al (2012) reported a more sustained release of loaded medium-chain fatty acids in nanoliposomes in comparison with our results. These differences in the release profile may be explained by the preparation method used (high-pressure microfluidization) [49]. In addition to the preparation method, the type of phospholipid used in liposome assembling is also effective in nanoliposome release behavior [50].

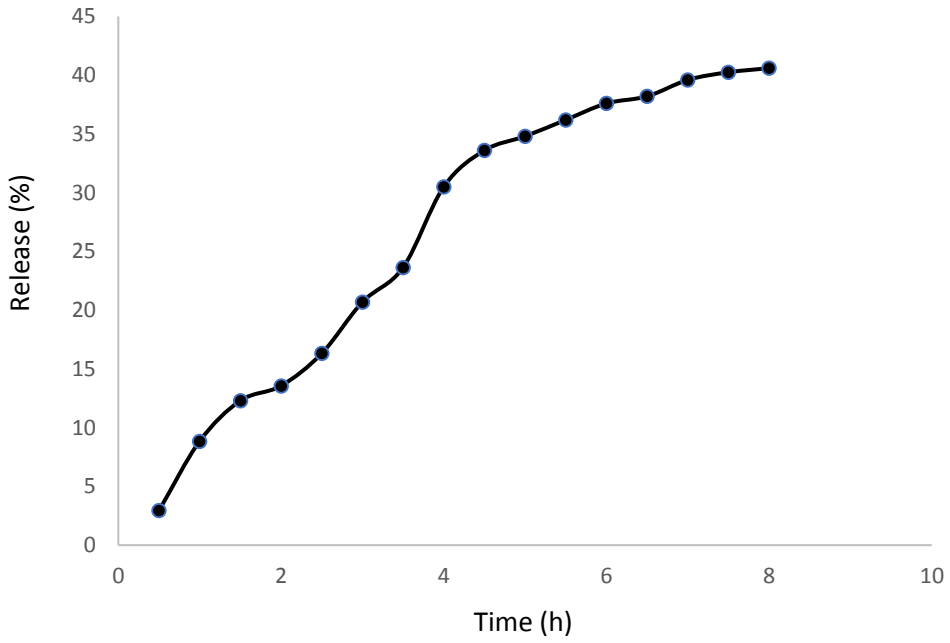


*Figure 3. Release profile of optimum nanoliposomes containing Padina algae extract.*

**CONCLUSIONS**

The bioactive properties of *Padina* algae extract were investigated and it was successfully encapsulated in nanoliposomes to improve its functionality. The optimal conditions were observed in the sample containing the lowest level of wall material (2.5% w/w) and the highest level of the algae extract (2%). Under these conditions, the particle size was 52.8 ± 0.52 nm (59.95 ± 0.19), and the zeta potential (-34.96 ± 0.89). The results related to particle size and zeta potential indicate long-term colloidal dispersion stability. The optimized nanoliposomes are continuously released over time under digestion conditions. In addition, this work, in addition to introducing algal *padina* extract as a biological compound, expands the knowledge of the in vitro digestive effects of liposomal nanoparticles containing *Padina* extract and supplies important information for designing suitable liposome formulations.

**Data Availability**

ALL DATA GENERATED OR ANALYZED DURING THIS STUDY ARE INCLUDED IN THE ARTICLE.

**CONFLICT OF INTEREST**

THE AUTHORS DECLARE NO CONFLICT OF INTEREST, FINANCIAL OR OTHERWISE.

**Author contribution**

Shila Safaeian and Rezvan Mousavi Nadoshan conceived and designed the study. Khadijeh Shirani Bidabadi performed research, analyzed data & writing. Nahid Rahimifard writing, review & editing.



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