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Research Article

Microencapsulation of Enriched Extracts of Two *Satureja* Species by Spray Drying, Evaluation of the Controlled Release Mechanism and Cytotoxicity

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Abstract

Background: Phenolic compounds are one of the main groups of secondary metabolites responsible for multiple biological and pharmacological properties that play a vital role in improving human health quality. Encapsulation by spray dryer creates protection toward the phenolic compounds as an efficient way for increasing product performance.

Methods: The phenolic compounds of *Satureja khuzistanica* Jamzad (SKH) and *S. rechingeri* Jamzad (SRH) were enriched based on adsorbent resin column chromatography and the enrichment index was confirmed by HPLC-UV analysis. Gum Arabic, carboxylated chitosan, and pectin with the optimum percentage of 1% w/w used to encapsulate SKH and SRH by the spray drying technique.

Results: Encapsulation yield was 38.18 – 59.00 %, particle size ranged 2.278 - 4.689 µm, and release time was between 4.08 - 82.08 min. The gum Arabic-based capsules showed the fastest and pectin-based revealed the slowest release time. The best kinetic model explained a release mechanism was Korsmeyer model. Anomalous transport was observed from all formulas except SKH-gum Arabic (case-I transport), SKH-pectin, and SRH-carboxylated-chitosan (super case-II transport). The cytotoxic activity of encapsulate SKH's revealed reducing the viability of AGS evaluated by the MTT compared with SRH's.

Conclusion: Encapsulation by spray drying has proven to be a promising technique to improve the bioavailability, release time, and mechanism of functional polyphenolic compounds as medicines, food supplements, and food additives.

Introduction

Nowadays, concerns related to whether a product is healthy, efficient, or not are always present. The demand for high-quality products with high nutritional and health value relates to the advances in quality improvements. Encapsulation by spray dryer converts a mixture of biopolymer and natural component/drugs to powder in a millisecond and produced the biopolymer-based encapsulated natural component/drugs. This technique improved stability and bioavailability of the natural product such as polyphenols, vitamins, and flavors or drugs like doxorubicin, diazepam, progesterone in a capsulated structure. Encapsulation protects the core substrate against decomposition by UV, heat, oxidation, evaporation and kept pharmacological properties at storage time or uptake by human cells. Furthermore, encapsulation technology could program delivery to target, enhance the releasing time, cover unpleasant tastes/flavors, reduce dosage, improve storage stability, and ease the handling.[1,2] The following methodologies have been developed for drying an active ingredient with various methods and several applications like pharmaceutical, food, cosmetics, and chemical industries.[2,3]

Meantime, the encapsulating agent (biopolymers) plays a critical role in the efficiency of the final product. Biopolymers are typically used as carriers in simple, dual, or matrix-based structures,[4] in which they are non-toxic, biodegradable, biocompatible and safe for human consumption. Also, these agents are selected according to the required site at gastronomical media and functionality of the final product.[5] Carboxylated chitosan (modified chitosan), gum Arabic, and pectin are three biopolymers

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used as natural encapsulation agents in current studies. Chitosan and modified-Chitosan (different types) are the second most abundant cationic natural-based biopolymers after cellulose. They are non-toxic, biodegradable, biocompatible, and have high film building capacity commonly used as an encapsulation agent.[6] Apple pectin is a linear non-toxic polysaccharide. It is characterized by extended retention time capacity, mainly due to the resistance to gastrointestinal conditions and lower solubility in acidic conditions. Generally, pectin improves the physicochemical stability of particles and enhances the release of natural compounds in a sustained or controlled way. These effects are notable about lipophilic compounds.[7] Gum Arabic extracted from *Acacia Senegal (L.)* with high molecular weight generally functions as a delivery carrier due to good film former, high encapsulation efficiency especially in high dosage, moisture stabilizer, suitable porous and easy to find and utilized.[8]

Besides, phenolic compounds are the secondary metabolites responsible for multiple biological and pharmacological properties. Consequently, these compounds exert antioxidative, anticarcinogenic,[9] antibacterial, antiviral, and anticancer activities.[10,11] The *Satureja* species belongs to the Lamiaceae family is a rich source of polyphenolic compounds.[12] *Satureja khuzistanica* Jamzad and *S. rechingeri Jamzad* are among the nine endemic species in fourteen indigenous *Satureja* species were found in Iran.[13] Furthermore, *Satureja* species are rich in specific metabolites, like terpenes, tannins, caffeic acid, and fatty acids.[14] These families are an abundant source of essential oils, particularly phenolic monoterpenes like carvacrol, thymol, and rosmarinic acid.[15]

Folk medical practices consider that *S. khuzistanica* can reduce cholesterol and blood pressure, control heart rate, rheumatic pain, and be a useful additive in food to reduce weight. Many researchers point towards the antimicrobial, anti-parasitic, and antioxidant properties of *S. khuzistanica* essential oil and its relation to the presence of carvacrol.[16] *S. khuzistanica* possesses antifungal, antimicrobial,[17] antioxidant, antidiabetic activities as well as hypoglycemic, anti-hyperlipidemic, anti-choleretic, anti-parasitic, anti-inflammatory, scolicidal, lysozyme activities and hematological factors,[18,19] Meanwhile, *S. rechingeri*[20] traditionally used to treat various diseases such as analgesic and antiseptic properties on healing activities.[13] *S. rechingeri* has been used in folk medicine to treat multiple diseases such as respiratory tract infections, diarrhea, urinary tract infections, and wound healing activities.[21] Moreover, many structural and biological similarities have been found between *S. khuzistanica, and S. rechingeri*[13], regarding anti-nociceptive, antioxidant, antidiabetic, anti-hyperlipidemic, anti-inflammatory activities of *S. rechingeri*[22], and *S. khuzistanica.*[23]

Concludingly, considering the structural and biological similarities between these two species, similar therapeutic effects should be observed, as well. Furthermore, the encapsulation of polyphenolic rich extracts (PEE) of *S. khuzistanica and S. rechingeri* by the spray drying technique could be good, protected candidate for a polyphenolic-rich additive in the food supplement or spice.

Materials and Methods

Materials

Rosmarinic acid was purchased from Sigma Aldrich. Ethanol was supplied from Valente e Ribeiro, Lda® (Alcanena, Portugal) with a purity of (99%). Apple pectin was purchased from Sigma Aldrich, with CAS N9000-69-5, from Switzerland. Gum Arabic deviated from the acacia tree (51201-1315371-24606P04) was supplied by Fluka (Germany). Modified chitosan (carboxylated chitosan) pharmaceutical-grade was purchased from China Eastar Group (Dongchen, China) (Batch no. SH20091010). Mettler Toledo AG245 analytical balance (Columbus, OH, USA) was used for technical weight measurement. The polystyrene adsorption resin, Diaion® HP-20, was purchased from Supelco (Bellefonte, PA, USA). HPLC grade solvents for chromatography were purchased from Scharlau (Barcelona, Spain). HPLC grade water was obtained by an EASY-pure II water purification system (Barnstead, Dubuque IA, USA).

Plants material and extraction procedure

Both species of *Satureja* were collected from the farm of Vasha Herbals Company at the flowering stage, Dezful, Khuzestan Province, Iran, and dried at ambient temperature. Plant materials have been characterized and voucher specimen (No. 58416 for *Satureja khuzestanica* Jamzad and No. 75587 for *Satureja rechingeri* Jamzad) were deposite at the Medicinal Plants and Drugs Research Institute (MPDRI), Shahid Beheshti University, Tehran, Iran.

A total of 300 g of dried leaves of each species were powdered and added to a 5L glass flask separately. The extraction was performed with a 3 L ethanol/water mixture (70:30 v/v). It was macerated for 24 hours and three times. Each time a new solvent was introduced into the maceration system, the process was repeated for three cycles. The solution was filtered after the maceration stage and concentrated at 40°C using a rotating vacuum evaporator. The dried extract was stored at 4°C before the next step.

The polyphenolic enrichment was performed by the Diaion® HP-20 adsorbent resin column (70 cm × 8 cm). The dried hydroethanolic extracts (50 g) were dissolved in distilled water (500 ml) and loaded on the resin column at a 5 ml/min flow rate. The polyphenolic compound were adsorbed by HP-20 resin; the column was washed with 5L of distilled water and the obtained solution was discarded. In the next step, the column of resin was eluted with ethanol (2 L) for desorption compounds. The desorbed solutions evaporated by a rotary evaporator in a vacuum and called polyphenolic enriched extracts (PEE). The PEEs were lyophilized and stored in the refrigerator for HPLC-UV analysis before proceeding to the next stage.[24,25]

The phytochemical analysis

The HPLC analysis was performed using a Waters liquid chromatography device consisting of a 2695 Separations Module (Milford, Massachusetts), an autosampler equipped with a 100 µl loop, and Photodiode Array Detectors (PDA) using 4.6 × 150 mm Sunfire ™ 3.5 µm C18 column. The mobile phase used to monitor a PEE was Methanol + 0.02% TFA / H_2O + 0.02% TFA. The following gradient was applied: 100% (A) and 0% (B) in time 0; 20% to 30% (A) in 10 min; 30% to 60% (A) in 30 min; 60% to 80% (A) in 10 min; 80% to 100% in 5 min; 100% (B) in 5 min; 100% to 20% in 10 min; all process took about 55 min, flow rate was 0.5 ml/min and the injection volume was 20 µL. The detection was performed at UV 254 and 366 nm. For all analytical HPLC analyses, the samples were dissolved in H_2O (10 mg/mL).

Rosmarinic acid was used as standard validation for HPLC analyses of PEE from *S. khuzistanica* and *S. rechingeri*. The calibration curve (y = 163038 x – 587108) was considered in the linear range (2-1000 µg/ml), detection limit (LOD, 0.02 µg/ml), quantification limit (LOQ, 0.2 µg/ml) with a correlation coefficient (r^2) of 0.9983.

The LC-ESIMS analysis was performed by A HPLC System with a quadruple pump connected to detector Photodiode (Agilent Waldbronn, Germany) coupled to the Bruker Esquire 3000, ion trap mass spectrometer with electrospray ionization (ESI) (Bruker Daltonic, Bremen, Germany). The HPLC separation condition was similar to the condition mentioned above.

Feed solutions of the spray-dryer

Gum Arabic, carboxylated chitosan, and apple pectin were used to encapsulate *S. khuzistanica* and *S. rechingeri* PEE by the spray drying technique. Each solution was prepared with an optimum percentage of 10% (w/v) of biopolymer, and an optimum 1% (w/v) of PEE in 100 mL of distilled water and stirred for two hours at room temperature. Two other solutions were prepared with a mixture of three biopolymers in which was added *S. khuzistanica*- and *S. rechingeri*-PEE, separately. Blank were prepared with varity of biopolymer (seperatly and complex) and used for comparison with experimental data. Biopolymer and PEEs were dissolved in distilled water and mixed by a magnetic stirrer to form a homogenized solutions.

Besides, all solutions were prepared and feed into a spray dryer. The active ingredient concentration was selected based on the amount of active compound in the extracts and the restrictions of the encapsulation procedure. The list of ingredients and the sample code were provided in Table 1. The characterization of microparticles was performed in terms of morphology, particle size and release profile.

Spray-drying conditions

Mini spray-dryer B-290 BÜCHI (Flawil, Switzerland) with a standard 0.5 mm nozzle was used for the spray drying technique. The encapsulation procedure was optimized beforehand based on previously reported data.[26] All solutions were prepared in deionized water, under adequate stirring conditions, at room temperature. The airflow rate was 35 m^3/h (90%), the solution flow rate was 3.5 mL/min, the inlet temperature was 115 °C, the air pressure was set to 5.0 bar, and 100% aspiration rate. The nozzle washer was set on three. Outlet temperature was changed due to several factors, such as the character of the solution and experimental conditions (typically around 60°C). The powders were collected in falcon tubes, protected in aluminum foil, and stored at 4°C.[27] The product yield (%) was calculated by total weight of microcapsule obtained from the spray dryer and the total mass of the initial feeding mixture, Equation 1.[28]

$$\text{Product yield (\%)} = \frac{\text{Total weight of gain powder (mg)}}{\text{Total weight of feed solution (mg)}} \times 100 \qquad \textbf{Eq. (1)}$$

Particle size distribution

Particle size was analyzed by laser granulometry using a Coulter counter-LS 230 Particle Size Analyser (Miami, FL, USA). The particle size distribution was analyzed in relative volume and relative numbers. By avoiding the unwanted agglomeration, the appropriate number of particles was suspended in ethanol before each measurement: the measurements were made in triplicate. The measurement method by Laser Diffraction and Polarization Intensity Differential Scattering (PIDS) took approximately 7 minutes for each run. The number distribution shows the most abundant particles with a specific size, while the volume distribution shows the particle size that contributes more strongly to the solution volume.

Scanning electron microscopy (SEM)

The scanning electron microscopy technique (SEM) was used to investigate the morphological analysis of the particles. The investigation was carried out in a JEOL JFC 100 apparatus at Centro de Materiais da Universidade do Porto (CEMUP). The process was performed under vacuum at 15 kV with a magnification of 100,000 – 30,000 for surface morphology investigation.

Controlled release experiments

Rosmarinic acid was used as a reference compound to validate the amount of PEEs release from microcapsule. The validation consisted of monitoring the release at 324 nm with a Thermo Scientific NanoDrop™ One C UV-vis spectrophotometer. The calibration curve was considered in the linear range, detection limit (LOD), quantification limit (LOQ). The evaluating of the release mechanism of *Satureja* species microcapsule provided essential data on microcapsules features. The release profile is a criterion for the active component is released from the capsule in the simulation area. Therefore, the control release experiment was performed in the water at a pH of 5.6. The rosmarinic acid calibration curve was y = 0.0298 + 26.11 x with a correlation coefficient (r^2) of 0.9979 and detection limit of 0.0011. By the way, 2 mg of encapsulated powder was suspended in water during a specific time. The specified

amount of standard released in a specified time was considered as a criterion amount of active ingredient entrapped in a capsule. The operation was done in triplicate under stirred at 60 rpm at room temperature and monitored at 324 nm.[29] The release mechanism was adapted according to the features of biopolymers[30] in which three different methods perform under the magnetic stirring condition at 25 ± 2°C. Therefore, the method's performance was evaluated by specific absorption of rosmarinic acid released from the biopolymer capsule over time. The mathematical model for release were designed by excel within a linear range of standards.

Release mechanism

Several parameters such as the preparation technique, the drying conditions, and the amount and feature of the biopolymer affected the release profile. A different statistical release model was designed to clarify the release profile of natural products.[31] Mathematical models are often valuable for predict tendencies, and some used repeated-measurement factors due to variance and standard deviation analysis. The literature reports three main categories of models to classify the release mechanism. Those are statistical, dependent, and independent models.[29] Depended models include several mathematical functions describing the release profile. The most popular statistical models are the zero-order, first-order, Hixson–Crowell, Baker–Lonsdale, Higuchi, Korsmeyer-Peppas, and Weibull models.[31] The ideal release mechanism of action follows a zero, half, or first-order kinetic model. Model-dependent methods are often used to access releasing mechanisms. A good releasing process may follow zero-order if the core contains single compounds, so a release occurs at a slow and constant rate.[32]

Usually, the releasing mechanisms are dependent on several factors. Therefore, the release of the core material should be more complicated than zero, half, and first orders. More sophisticated mathematical models like Korsmeyer-Peppas are frequently related to the kinetic of potent compounds and used to validate the current sample in this study.

$$Qt/Q\infty = K_K t^n \qquad \text{Eq. (2)}$$

$Qt/Q\infty$ presents the amount of active compound released until time t, K_K is the Korsmeyer constant, n clarify a drug transport mechanism, and t issued a rate as a function of time. The microcapsules assume a spherical geometry; when n < 0.43, drug transport occurs by diffusion following Fick law (case-I transport). When 0.43 < n < 0.85, drug transport is considered anomalous, involving a combination of diffusion and swelling release. If n = 0.85, drug transport follows a zero-order release (case-II transport). Super case-II transport (polymer matrix relaxation) occurs when n > 0.85.[32,33]

Cytotoxicity assay

Extracts were prepared in culture media and tested in the concentration range of 31.25 - 500 µg/ml. Cells were exposed to the extracts for 24 hours, after which the MTT reduction assay was performed. The test was done on human gastric cancer cell line AGS (1.5×10^4 cells/well) and lung cancer cell line A549 (1.5×10^4 cells/well), as evaluated by the MTT assay. [34,35] Several researchers investigated the safety assessment of these samples in normal cell lines and it was concluded that *S. khuzistanica* and *S. rechingeri* had been assessed *in vivo* in multiple species, with no adverse effects have been described.[36,37]

Results

Phytochemical profiling

The analysis of the phytochemical profile of PEEs of *S. khuzistanica and S. rechingeri* is represented in Figure 1. Rosmarinic acid, one of the principal compounds, showed a peak in the HPLC chromatogram (retention time of 29.5 minutes) in both species (Figure 1). The LC-MS analysis in negative mode and UV spectrum confirmed the presence of rosmarinic acid in both species with *m/z* at 359 $[M-H]^-$ and 719 $[2M-H]^-$, the two other major compounds in the extrat identified by ESI-MS showed a quasi-molecular ion peak at *m/z* 593 $[M-H]^-$ for apigenin-7-*O*-gentiobioside and *m/z* 717 $[M-H]^-$ for epi-salvianolic acid B (Figure 2). The HPLC chromatograms for extracts before and after the enrichment process with a resin have an identical retention time. The data indicate that enrichment does not modify the phytochemical profile of the extracts qualitatively. Diaon HP-20 resin column chromatography significantly adsorbed and enriched polyphenolic compounds and

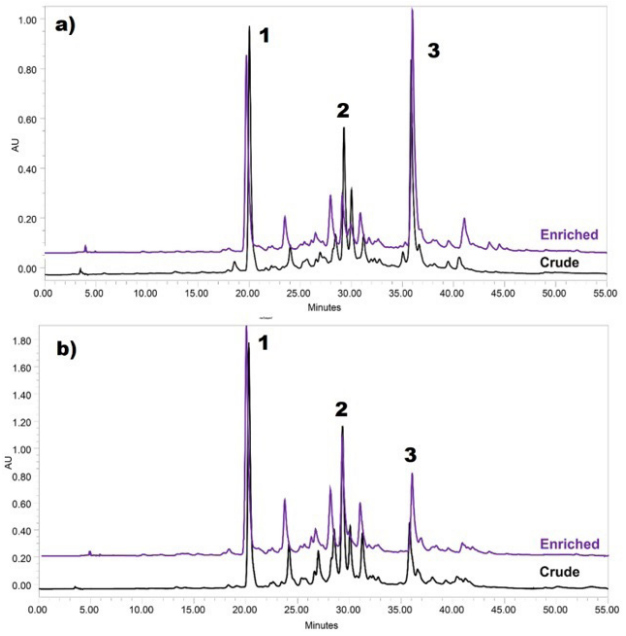

Figure 1. Comparison of crude and polyphenolic enrich HPLC chromatograms of *S. khuzistanica* (A), *S. rechingeri* (B) at a wavelength of 324 nm. Retention time 20.00; apigenin-7-*O*-gentiobioside (**1**), retention time 29.50; rosmarniric acid (**2**), retention time 36.00; epi-salvianolic acid B (**3**).

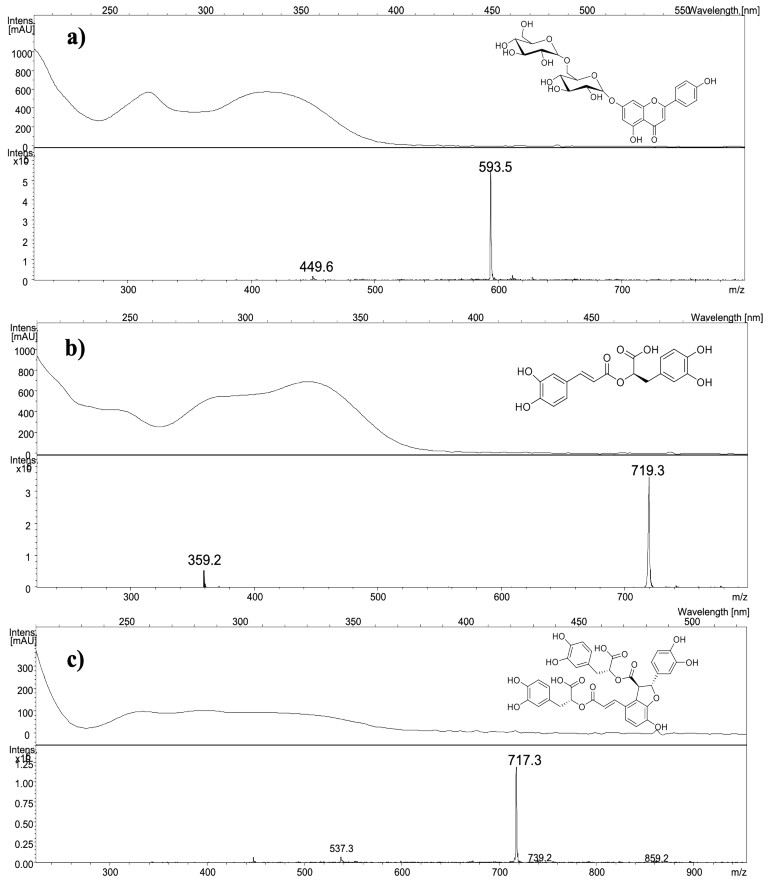

Figure 2. The UV spectrum of rosmarinic acid (above) and mass spectra of rosmarinic acid in negative mode (below). Image (a); apigenin-7-*O*-gentiobioside, (b); rosmarniric acid, (c); epi-salvianolic acid B.

suppressed undesirable compounds such as sugars and chlorophyll. The rosmarinic acid contents in *S. khuzistanica* crude extract (CE) and PEE were quantified as 29.90 mg/L and 54.03 mg/L, respectively. Regarding *S. rechingeri* the rosmarinic acid content was 51.27 mg/L and 98.04 mg/L in the CE and PEE, respectively. The enrichment process resulted in an increase from 1.8 and 1.9 – related to *S. khuzistanica* and *S. rechingeri*, respectively (Figure 1).

Product yield

Particle yield depends on several parameters influencing the spray drying process, such as the solution features, inlet, outlet temperature, and pressure.[38] The product yield ranged between 38.18% - 59.00 %, including full and free microparticles (Table 1). The unloaded capsule presented a higher result, around 49.00– 58.67 %, while the loaded one presented a yield of approximately 38.18 – 59.00 %. Finally, the lowest and the highest yields were reported for SRH-P and SKH-MCh microparticles. Particle yield directly depended on the condition of the equipment and or loading conditions. It was not related to the biopolymer features. Table 1 shows the product of all samples in detail.

Microcapsules size distribution

The evaluation of particle size distribution in relative volume and number carried out in samples dispersed in ethanol (Table 2). The particle size distributions in the relative of number (independently) and volume (independently) were similar and uniform regarding the feature of related biopolymer and heterogeneous in terms of comparison between the type of biopolymer, Table 2. According to each biopolymer's relative volume and relative number, the monodisperse in size distribution was noticeable. The volume distribution of these biopolymers was compared to other biopolymers as follows; the largest particle size was observed for the pectin capsules, about 3.101 and 3.274 µm to SKH-P and SRH-P, respectively. Whether in empty or full capsules, pectin-based particles showed a size distribution ranging between 97 - 105 nm in number. Modified chitosan-based capsules also exhibited a similar size for loaded particles. In this case, the averages were 2.278 and 2.34 µm to SKH-MCh and SRH-MCh, respectively. SKH-MCh and SRH-MCh revealed particle size ranged between 789 - 759 nm in number and 2.278 - 2.34 µm in volume, respectively. The gum Arabic-based capsules showed almost the same average size in volume. Those values were 2.622 and 2.661 µm related to SKH-GA and SRH-GA, respectively. Although the unloaded gum Arabic-based capsules size was about 3.568 in volume, these particles presented a similar size compared to the loaded one, ranging between 79 nm in number. Briefly, according to the particle size listed in Table 2, it is clear that the modified chitosan gives the bigger particle in number, opposite to the smallest particles were shown in relative volume.

Table 1. Product yield and Size distribution in number and volume listed utilizing the empty capsule and *S. khuzistanica* and *S. rechingeri* microcapsules.

Microcapsule content	Sample name	Sample code	Yield %	Volume (µm)			Number (µm)			Total PDI
				Mean	SD	PDI	Mean	SD	PDI	
Empty biopolymer	Gum Arabic	GA	53.00	3.568	2.384	1.593	0.097	0.091	0.085	0.839
	Modified chitosan	MCh	49.00	4.292	3.344	2.605	0.153	0.226	0.334	1.470
	Pectin	P	54.00	4.689	4.483	4.286	0.097	0.091	0.086	2.186
	Mix-biopolymers	Mix-b	58.67	2.869	1.797	1.126	0.099	0.097	0.095	0.610
Plant extracts	*S. khuzistanica*-Gum Arabic	SKH-GA	50.00	2.622	1.554	0.921	0.115	0.141	0.173	0.547
	S. khuzistanica-Modified chitosan	SKH-MCh	59.00	2.278	1.261	0.698	0.789	0.508	0.327	0.513
	S. khuzistanica-Pectin	SKH-P	51.82	3.101	2.114	1.441	0.104	0.115	0.127	0.784
	S. khuzistanica-Mix biopolymers	SKH-Mix-b	57.58	2.759	1.690	1.035	0.112	0.134	0.160	0.598
	S. rechingeri-Gum Arabic	SRH-GA	38.18	2.661	1.596	0.957	0.132	0.175	0.232	0.595
	S. rechingeri-Modified chitosan	SRH-MCh	42.73	2.340	1.339	0.766	0.759	0.502	0.332	0.549
	S. rechingeri-Pectin	SRH-P	38.18	3.274	2.577	2.028	0.105	0.118	0.133	1.080
	S. rechingeri-Mix biopolymers	SRH-Mix-b	53.94	2.684	1.703	1.081	0.139	0.184	0.244	0.662

*PDI; Polydispersity (dispersity) is a factor in which describe the degree of non-uniformity or heterogeneity of size distribution. The values < 0.05 indicate highly monodisperse and the values > 0.7 indicate extremely broad particle size distribution.

Additionally, both number and volume distributions showed that unloaded biopolymers particle size is higher than the loaded ones. Unloaded biopolymer particles had an average size of 2.869 to 4.689µm in volume and 99 to 153 nm in number. The following parameter may be due to adhesion (might affinity) of the particle to biopolymer in which they created a semi-stable colloid in solution. Therefore, the semi-stable colloids in solution were passed the spray dryer nozzle in colloids form, making the particle smaller. Furthermore, the variation in size was due to several parameters such as type of biopolymer, the viscosity of feed solution, and drying condition.

SEM images and morphology

SEM images of microparticles obtained by spray drying are shown in Figure 3. SEM images showed a potential difference in the shape and morphology of particle surface according to the type of biopolymer. Samples using modified chitosan showed a smooth, circular, round, and spherical surface morphology without any wrinkle and edge. The pectin sample showed semi-cubes and an unfair morphology with lots of pores and cavities. Gum Arabic particle shape was similar to pectin, but with more wrinkle and edge, with a gap between winkle on the cubic surface of the particle (Figure 3).

Stabilization/ release time

Rosmarinic acid was used as a reference compound for the monitoring release process. The amount of rosmarinic acid released from the capsules during a specific time

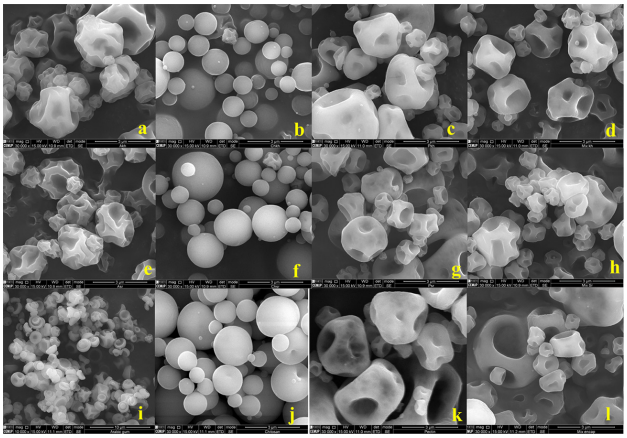

Figure 3. SEM images of microcapsule of SKH-GA (a). SKH-MCh (b), SKH-P (c), and MIX of three Mix-SKH (d), SRH-GA (e). SRH -MCh (f), SRH -P (g), and MIX of three "Mix-SRH"(h), and empty biopolymers gum Arabic (I), modified Chitosan (m) and Pectin (n) and mix of three biopolymers (o), Magnification 30000, beam intensity (HV) 15.00 kV, the distance between the sample and the lens (WD) around 10 mm.

was used as the standard percentage of the extract released from capsules. The stabilization time of three different biopolymers is shown in Figure 4. Pectin-based microcapsule revealed a similar release behavior in both species, with stabilization times around 82.08 and 81.08 for SKH-P and SRH-P, respectively (Table 2), which was presented as the most extended release times among other samples. Gum Arabic-based offers the fast release of about 7.33 and 4.08 min for SKH-GA and SRH-GA, respectively. The stabilization time of active compounds loaded on the

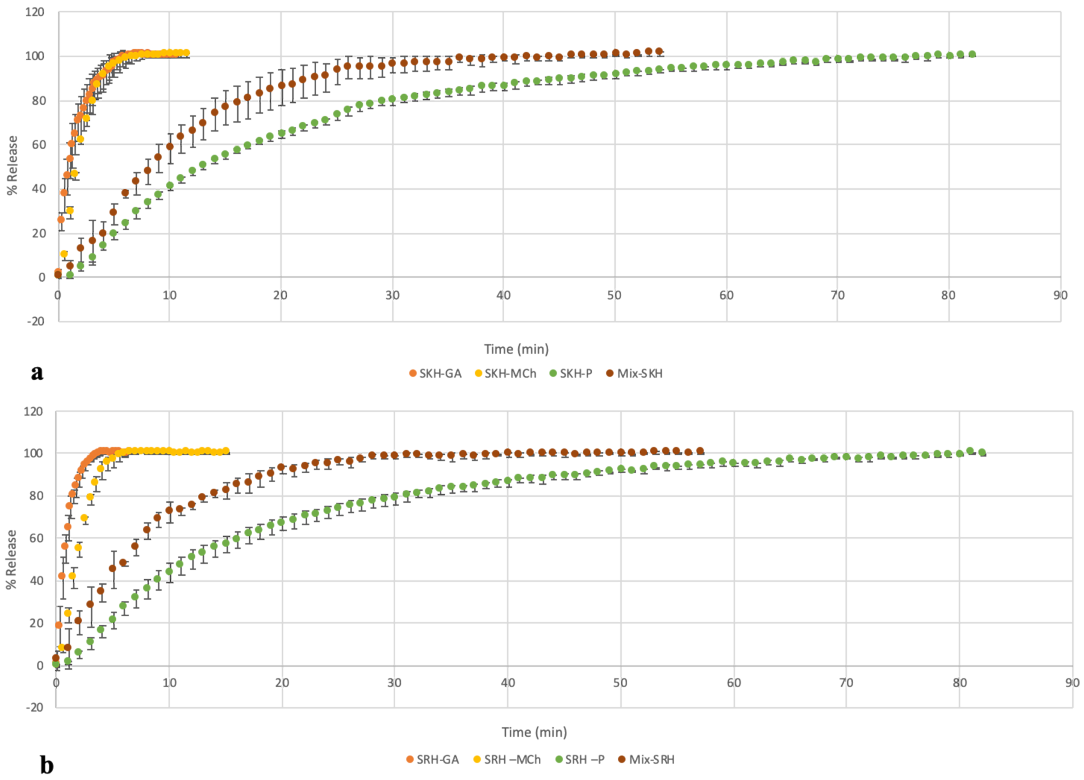

Figure 4. Release mechanism profiles and stabilization time were obtained for *S. Khuzistanica* (a), and *S. Rechingeri* (b). Results are presented as the average and standard deviation of three independent assays.

modified chitosan was 8.58 and 7.58, belong to SKH-MCh and SRH-MCh, respectively.

In conclusion, modified chitosan and gum Arabic-based microparticles presented a similar and slowest stabilization time, approximately less than 8.58 min. In contrast, pectin-based macroparticles had the longest stabilization time (more than 82.08 min) Table 2, Figure 4. It must conclude that the type of biopolymer directly affected the release time of encapsulated *Satureja* species.

Kinetic model

New generations of delivery systems directly depended on the type of carriers and dosage of active part. The mathematical models are applied using repeated-measurement factors to estimate the release profile by the kinetic model. Korsmeyer-PPEEas model was introduced as the most suitable kinetic model to describe the release mechanism of particles loaded with natural active compounds on a matrix-based and straightforward delivery system. Matrix-based particles loaded with herbal extracts seem to present an excellent release mechanism.[31] Thus, the correlation coefficients (r^2) obtained from the Korsmeyer-PPEEas model involving microcapsules containing *S. khuzistanica* and *S. rechingeri* coated by gum Arabic, modified chitosan, and pectin as a carrier, represented between 0.9665 and 0.9965. The release mechanisms are shown in detail in Table 2. The values of the parameter (n) in the Korsmeyer-PPEEas model shows that the release of *Satureja* species from the capsule is controlled mainly by anomalous transport, involving a combination of diffusion and swelling release named SKH-MCh, SRH-GA, and SRH-P. Mix-SKH and Mix-SRH

Table 2. Parameters and correlation coefficients (Korsmeyer kinetics) were applied to the experimental release profiles.

Sample	Release Stable Time (min)	R^2	K_k (min-n)	n			
				n < 0.43	0.43 < n < 0.85	n = 0.85	n > 0.85
SKH-GA	7.330	0.9821	0.9086	0.0186	-	-	-
SKH-MCh	8.580	0.9846	0.6132	-	0.3204	-	-
SKH-P	82.08	0.9950	0.0480	-	-	-	0.9527
Mix-SKH	44.08	0.9665	0.1386	-	0.6150	-	-
SRH-GA	4.080	0.9965	0.7121	-	0.5358	-	-
SRH -MCh	7.580	0.9536	0.1912	-	-	-	1.2031
SRH -P	81.08	0.9856	0.0694	-	0.7824	-	-
Mix-SRH	35.08	0.9841	0.2256	-	0.4853	-	-

*Stable time is a time when the release start to be stable and the curve change to linear.

(mix of three biopolymers). Furthermore, SKH-GA release occurs by diffusion following Fick law (case-I transport), and SKH-P and SRH-MCh release follow super case-II transport (polymer matrix relaxation) (Table 2).

Cytotoxicity activity

Polyphenolic extracts were prepared in culture media and tested in the concentration range of 31.25 - 500 µg/mL. Cells were exposed to the different extracts for 24 hours, after which the MTT reduction assay was performed. *S. khuzistanica* elicited a significant decrease in viability towards the human gastric cancer cell line AGS in a concentration as low as 125 µg/mL, the same being true for *S. rechingeri*. In the A549 cell line, *S. khuzistanica* exhibited a similar capacity, albeit to a lower extent. In contrast, only the highest concentration of *S. rechingeri*, 500 µg/mL, affected the viability adversely (Figure 5).

Discussion

Natural compounds play an essential role in human health with beneficial effects on the recovery of several diseases. Flavonoids and caffeic acids are the principal polyphenol structural groups identified in S. *khuzistanica* PEE. Rosmarnic acid was used as a standard and was detected at a retention time of 29.5 min on the HPLC chromatogram previously identified by this species.[39,40] Meanwhile, the *m/z* at 359 $[M-H]^-$ and 719 $[2M-H]^-$ in the mass spectra determined the existence of rosmarnic acid with a molecular weight of 360, which is in agreement with Fatemi and coworkers 2019.[41] Due to the probable decomposition of the phytoconstituent in the human body, a spray drying encapsulation technique has been developed to improve the protection of the phytoconstituent.[42] Gum Arabic, modified chitosan, and pectin were chosen due to inherent characteristics such as process stability, low hygroscopicity, appropriate film-forming, and an appropriate release profile.[32] As a result, the critical parameter like choosing a right encapsulating agent, optimized preparation method and optimize drying condition was presented in this study, which was consistent with the fact of delivery in literature.[43-47] As mentioned in the results, the highest yield was observed from modified chitosan, within a range of values reported by the literature in nearly the same condition with a spray dryer (30 - 75%).[43] Besides, our findings are in acceptance with other studies in the area of surface morphology. For instance, it was reported that the modified carboxylation chitosan (water-soluble) had a surface round and spherical morphology.[44] The smooth and spherical morphology has been reported from modified hydrosoluble carboxylated chitosan loaded with vitamin B12. A semi-cubic unfair surface was observed for pectin while an irregular cubic surface with numerous wrinkles and spherical edges was observed from gum Arabic particles before.[26]

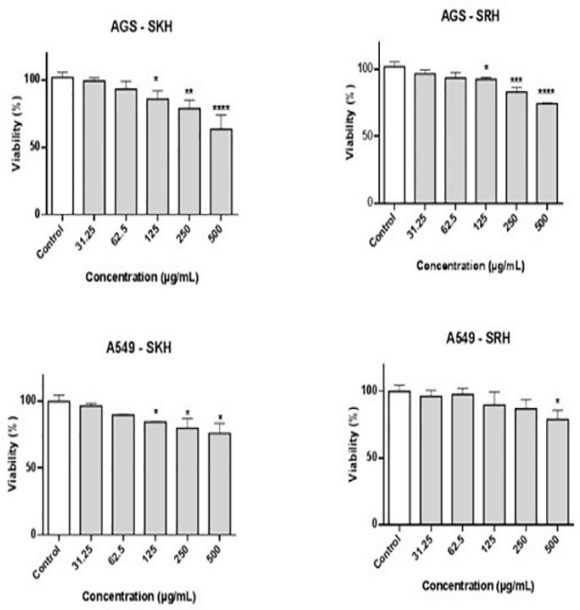

Figure 5. Effect of aqueous extracts upon the viability of the human gastric cancer cell line (AGS) and the human lung cancer cell line (A549) are evaluated by the MTT assay. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Finally, it was concluded that the type of biopolymer directly influences the form and morphology of microcapsules. Due to the stability of the microparticles, several studies were conducted using a similar kind of particles but containing vitamins (B1, B12, A) and retinoic acid. These studies confirmed that the particles stored for at least four months were stable. Therefore, it was concluded that the bioactive compounds within the particulates remain active after this storage period, without degradation for at least four months.[26,45]

The effects of biopolymers on the release time have already been reported in other studies.[46] Olive leaf extracts are a source of polyphenolic compounds, when encapsulated by chitosan, present a similar stabilization release time compared to our results.[47] Moreover, research has shown that when pectin is used as a carrier, the release time of curcumin nanoparticles increases significantly.[48] However, a kinetic model was used to describe the release of carriers on a routine basis. For instance, a gelatine microsphere loaded with trans-retinoic followed a zero-order or Higuchi kinetics.[49] Korsmeyer model was used to explain the release mechanism of hydrogel pH-sensitive particles.[50] As well, the Korsmeyer model was used to describe the release profile of chitosan-based nanoparticles.[51] In a convincing way, different kinetic models open a wise view of the release functionality. Therefore, researchers have found appropriate and controllable delivery systems and release mechanisms to be validated for the application in pharmaceutics medicine, chemotherapy, food, and supplement, and cosmetic and skincare product with the help of a kinetic model in case of in vitro release assay.

Conclusion

Conclusionally, our study suggests a new delivery system for two encapsulated Iranian endemic *Satureja* species, *S. khuzistanica* and *S. rechingeri,* as a function of food

spice and dietary food supplements. The pectin-based formula showed the largest particle size between 3.101 and 3.274 µm with a product yield of 51.82 and 38.18, belong to SKH-P and SRH-P, respectively. Moreover, the slowest stabilize time was observed from pectin-based microcapsule around 82.08 min to SKH-P and 81.08 min to SRH-P. The fastest stabilize time was observed from SRH-GA microcapsule in 4.08 min with 2.661 µm particle size, 38.18% particle yield, and anomalous (combination of diffusion and swelling release) release mechanism. Koresmeyer-Peppas was the kinetic model matched the release mechanism profile.

Author Contributions

FF: Experimental part, writing the manuscript; SNE: Supervision, experimental validation in phytochemical part, developing the draft of the paper; DMP: Biological assessment; BNE and FR: Supervision of encapsulation part and developing the draft of the article. Authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors report no conflicts of interest.

References

1. Comunian TA, Favaro-Trindade CS. Microencapsulation using biopolymers as an alternative to produce food enhanced with phytosterols and omega-3 fatty acids: A review. Food Hydrocoll. 2016;61:442-57. doi:10.1016/j.foodhyd.2016.06.003
2. Ré M-I. Formulating drug delivery systems by spray drying. Dry Technol. 2006;24(4):433-46. doi:10.1080/07373930600611877
3. Jafari SM, He Y, Bhandari B, Jafari SM, He Y, Bhandari B. Encapsulation of nanoparticles of d-limonene by spray drying: role of emulsifiers and emulsifying techniques. Dry Technol. 2010;25(6):1069-79. doi:10.1080/07373930701396758
4. Robert P, Gorena T, Romero N, Sepulveda E, Chavez J, Saenz C. Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. Int J Food Sci Technol. 2010;45(7):1386-94. doi:10.1111/j.1365-2621.2010.02270.x
5. Rui Xiong, Anise M.Grant, Ruilong Ma, Shuaidi Zhang, Vladimir V. Tsukruk. Naturally-derived biopolymer nanocomposites: Interfacial design, properties and emerging applications. Mater Sci Eng R Rep. 2018;125:1-41. doi:10.1016/j.mser.2018.01.002
6. Bernkop-Schnürch A, Dünnhaupt S. Chitosan-based drug delivery systems. Eur J Pharm Biopharm. 2012;81(3):463-9. doi: 10.1016/j.ejpb.2012.04.007
7. Chang C, Wang T, Hu Q, Zhou M, Xue J, Luo Y. Pectin coating improves physicochemical properties of caseinate/zein nanoparticles as oral delivery vehicles for curcumin. Food Hydrocoll. 2017;70:143-51. doi:10.1016/j.foodhyd.2017.03.033
8. Arepally D, Goswami TK. Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. LWT. 2019;99:583-93. doi:10.1016/j.lwt.2018.10.022
9. Vaher M, Koel M. Separation of polyphenolic compounds extracted from plant matrices using capillary electrophoresis. J Chromatogr A. 2003;990(1–2):225-30. doi:10.1016/S0021-9673(02)02013-7
10. Soobrattee MA, Bahorun T, Aruoma OI. Chemopreventive actions of polyphenolic compounds in cancer. BioFactors. 2006;27(1-4):19-35. doi:10.1002/biof.5520270103
11. Cai Y, Evans FJ, Roberts MF, Phillipson JD, Zenk MH, Gleba YY. Polyphenolic compounds from *Croton lechleri*. Phytochemistry. 1991;30(6):2033-40. doi:10.1016/0031-9422(91)85063-6
12. Dardioti A, Karousou R, Lanaras T, Kokkini S. Diversity of *Satureja pilosa* subsp. origanita essential oils: A new " Oregano" from East Mediterranean. Biochem Syst Ecol. 2012; 40:178-83. doi:10.1016/j.bse.2011.10.015
13. Nooshkam A, Mumivand H, Hadian J, Alemardan A, Morshedloo MR. Drug yield and essential oil and carvacrol contents of two species of Satureja (*S. khuzistanica* Jamzad *and S. rechingeri* Jamzad) cultivated in two different locations. J Appl Res Med Aromat Plants. 2017;6:126-30. doi:10.1016/j.jarmap.2017.04.002
14. Momtaz S, Abdollahi M. An update on pharmacology of satureja species; from antioxidant, antimicrobial, antidiabetes and anti-hyperlipidemic to reproductive stimulation. Int J Pharmacol. 2010;6(4):346-53. doi:10.3923/ijp.2010.346.353
15. Hajimehdipoor H, Saeidnia S, Gohari A, Hamedani M, Shekarchi M. Comparative study of rosmarinic acid content in some plants of Labiatae family. Pharmacogn Mag. 2012;8(29):37-41. doi:10.4103/0973-1296.93316
16. Haeri S, Minaie B, Amin G, Nikfar S, Khorasani R, Esmaily H, et al. Effect of *Satureja khuzestanica*

essential oil on male rat fertility. Fitoterapia. 2006;77(7-8):495-9. doi: 10.1016/j.fitote.2006.05.025
17. Mazarei Z, Rafati H. Nanoemulsification of *Satureja khuzestanica* essential oil and pure carvacrol; comparison of physicochemical properties and antimicrobial activity against food pathogens. LWT. 2019;100:328-34. doi:10.1016/j.lwt.2018.10.094
18. Jafari F, Ghavidel F, Zarshenas MM. A critical overview on the pharmacological and clinical aspects of popular satureja species. JAMS J Acupunct Meridian Stud. 2016;9(3):118-27. doi:10.1016/j.jams.2016.04.003
19. Giannouli E, Athanasopoulos D, Panagopoulos G, Tsiara S, Karageorgiou CE. *Paraneoplasmatic encephalitis* due to Merkel cell neuroendocrine skin carcinoma. J Neurol Sci. 2013;333:e703. doi:10.1016/j.jns.2013.07.2427
20. Jamzad Z. *Satureja rechingeri* (Labiatae)—a new species from Iran. Ann Naturhist Mus Wien. 1996;98:75-7.
21. Hadian J, Esmaeili H, Nadjafi F, Khadivi-Khub A. Essential oil characterization of *Satureja rechingeri* in Iran. Ind Crops Prod. 2014;61: 403-9. doi:10.1016/j.indcrop.2014.07.034
22. Alizadeh A. Essential oil composition, phenolic content, antioxidant, and antimicrobial activity of cultivated *Satureja rechingeri Jamzad* at different phenological stages. J Biosci. 2015;70:51-8. doi:10.1515/znc-2014-4121
23. Mirjalili MH, Kanani MR, Salehnia A, Ganjipoor P, Phytochemical and Morphological Characterization of *Satureja khuzistanica* Jamzad Populations from Iran. Chem Biodivers. 2011;8:902-15. doi:10.1002/cbdv.201000249
24. Ebrahimi SN, Gafner F, Dell'Acqua G, Schweikert K, Hamburger M. Flavone 8-C-glycosides from *Haberlea rhodopensis* Friv. (Gesneriaceae). Helv Chim Acta. 2011;94(1):38-45. doi:10.1002/hlca.201000378
25. Shehzad O, Jin Ha I, Park Y, Wan Ha Y, Shik Kim Y. Development of a rapid and convenient method to separate eight ginsenosides from Panax ginseng by high-speed counter-current chromatography coupled with evaporative light scattering detection. J Sep Sci. 2011; 34(10):1116-22. doi:10.1002/jssc.201000932
26. Gonçalves A, Estevinho BN, Rocha F. Design and characterization of controlled-release vitamin A microparticles prepared by a spray-drying process. Powder Technol. 2017;305: 411-7. doi:10.1016/j.powtec.2016.10.010
27. Kha TC, Nguyen MH, Roach PD, Stathopoulos CE. Microencapsulation of Gac oil: Optimisation of spray drying conditions using response surface methodology. Powder Technol. 2014;264: 298-309. doi:10.1016/j.powtec.2014.05.053
28. Santana AA, Oliveira RA de, Kurozawa LE, Park KJ. Microencapsulation of pequi pulp by spray drying: use of modified starches as encapsulating agent. Eng Agríc. 2014;34(5):980-91. doi:10.1590/S0100-69162014000500017
29. Ito R, Golman B, Shinohara K. Controlled release with coating layer of permeable particles. J Control Release. 2003;92:361-8. doi:10.1016/S0168-3659(03)00363-8
30. Ziaee M, Moharramipour S, Mohsenifar A. MA-chitosan nanogel loaded with Cuminum cyminum essential oil for efficient management of two stored product beetle pests. J Pest Sci. 2014;87(4):691-9. doi:10.1007/s10340-014-0590-6
31. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67(3):217-23.
32. Pothakamury UR, Barbosa-Cánovas G V. Fundamental aspects of controlled release in foods. Trends Food Sci Technol. 1995;6(12):397-406. doi:10.1016/S0924-2244(00)89218-3
33. Unagolla JM, Jayasuriya AC. Drug transport mechanisms and in vitro release kinetics of vancomycin encapsulated chitosan-alginate polyelectrolyte microparticles as a controlled drug delivery system. Eur J Pharm Sci. 2018;114:199-209. doi:10.1016/j.ejps.2017.12.012
34. Videira RA, Andrade PB, Monteiro LS, Valentão P, Ferreira PMT, Pereira DM. Toxicity and structure-activity relationship (SAR) of α,β-dehydroamino acids against human cancer cell lines. Toxicol In Vitro. 2018;47:26-37. doi:10.1016/j.tiv.2017.10.027
35. Figueiredo-González M, Valentão P, Pereira DM, Andrade PB. Further insights on tomato plant: Cytotoxic and antioxidant activity of leaf extracts in human gastric cells. Food Chem Toxicol. 2017;109:386-92. doi:10.1016/j.fct.2017.09.018
36. Rasooli A, Fatemi F, Akbarzadeh K, Dini S, Bahremand S. Synergistic protective activity of deuterium depleted water (DDW) and *satureja rechingeri* essential oil on hepatic oxidative injuries induced by acetaminophen in rats. J Essent Oil Bear Plants. 2016;19(5):1086-101. doi:10.1080/0972060X.2015.1111776
37. Sahraei H, Gharzi A, Amiri H, Abbasi M, Gholami M. Wound healing effect of satureja khuzistanica and satureja rechingeri ethanolic extracts in NMRI adult mice. Zahedan J Res Med Sci. 2016 16;18(5): e6665. doi:10.17795/zjrms-6665
38. Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Res Int. 2007;40(9):1107-21. doi:10.1016/j.foodres.2007.07.004
39. Davoodi M, Rustaiyan A, Ebrahimi SN. Monoterpene flavonoid from aerial parts of *Satureja khuzistanica*. Rec Nat Prod. 2017;12(2):175-8. doi:10.25135/rnp.19.17.06.109
40. Siavash Saei-Dehkordi S, Fallah AA, Heidari-Nasirabadi M, Moradi M. Chemical composition, antioxidative capacity and interactive antimicrobial potency of *Satureja khuzestanica Jamzad* essential oil and antimicrobial agents against selected food-related microorganisms. Int J Food Sci

Technol. 2012;47(8):1579-85. doi:10.1111/j.1365-2621.2012.03006.x
41. Fatemi F, Abdollahi MR, Mirzaie-asl A, Dastan D, Garagounis C, Papadopoulou K. Identification and expression profiling of rosmarinic acid biosynthetic genes from *Satureja khuzistanica* under carbon nanotubes and methyl jasmonate elicitation. Plant Cell Tissue Organ Cult. 2019;136(3):561-73. doi:10.1007/s11240-018-01537-8
42. Raman RK, Santhalakshmy S, Bosco SJD, Deshwal GK. Effect of encapsulating agents on antioxidative properties of spray dried Jamun juice powder. Int J Chem Stud. 2020;8(3):1807-10. doi:10.22271/chemi.2020.v8.i3y.9468
43. Mourya VK, Inamdar NN. Chitosan-modifications and applications: Opportunities galore. React Funct Polym. 2008;68(6):1013-51. doi:10.1016/j.reactfunctpolym.2008.03.002
44. Estevinho B N. Damas A M., Martins P. Rocha F. The influence of microencapsulation with a modified chitosan (water soluble) on β-galactosidase activity. Dry Technol. 2014;32:1575-86. doi: 10.1080/07373937.2014.909843
45. Carlan IC, Estevinho BN, Rocha F. Study of microencapsulation and controlled release of modified chitosan microparticles containing vitamin B12. Powder Technol. 2017;318:162-9. doi: 10.1016/j.powtec.2017.05.041
46. Ersus S, Yurdagel U. Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota L.*) by spray drier. J Food Eng. 2007;80(3):805-12. doi:10.1016/j.jfoodeng.2006.07.009
47. Kosaraju SL, D'ath L, Lawrence A. Preparation and characterisation of chitosan microspheres for antioxidant delivery. Carbohydr Polym. 2006;64(2):163-7. doi:10.1016/j.carbpol.2005.11.027
48. Nguyen AT, Winckler P, Loison P, Wache Y, Chambin O. Physico-chemical state influences in vitro release profile of curcumin from pectin beads. Colloids Surfaces B Biointerfaces. 2014;121:290-8. doi: 10.1016/j.colsurfb.2014.05.023
49. Dinarvand R, Rahmani E, Farbod E. Gelatin microspheres for the controlled release of all-trans-retinoic acid topical formulation and drug delivery evaluation. Iran J Pharm Res. 2010;2(1):47-50. doi:10.22037/ijpr.2010.35
50. Gupta P, Vermani K, Garg S. Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov Today. 2002;7(10):569-79. doi:10.1016/S1359-6446(02)02255-9
51. Soares PIP, Isabel A, Carvalho J, Ferreira IMM, Novo CMM, Paulo J. Chitosan-based nanoparticles as drug delivery systems for doxorubicin: Optimization and modelling. Carbohydr Polym. 2016;147: 304-12. doi:10.1016/j.carbpol.2016.03.028.