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Comparative Study on Preparation, Characterization and Antioxidant Activity of Encapsulated *Viola odorata* L. Extract Based on Gum Arabic-Gelatin and *Lepidium Perfoliatum* L. Seed Gum Nanoemulsions

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Abstract

Viola odorata L. (Sweet violet) has been known with various biologic activities due to its secondary metabolites including flavonoids, glycosides, alkaloids, tannins and saponins. Nanoencapsulation of its extract can be an effective approach to improve the pharmaceutical application of Sweet violet phytochemicals. This study aimed to prepare encapsulated violet extract by two encapsulation methods and compare the antioxidant activity, and evaluate the encapsulation efficiency and physicochemical properties. The extract was encapsulated by double nanoemulsion (gum Arabic-gelatin complex) and nanoemulsion (Lepidium perfoliatum seed gum) methods and using the spray and freeze-drying processes. As a result, the highest encapsulation efficiency (85.82%) and more than antioxidant activity (136.67%) was in double nanoemulsion with freeze-drying. Nanoemulsions showed a higher stability index (97.75%) and greater emulsifying ability (99.84%) than double nanoemulsion due to higher zeta potential. . Due to Dynamic light scattering analysis, the nanoemulsion particle size (306.5 nm) was smaller than that of double nanoemulsion (798.3 nm). Density and moisture content of the double emulsion with freeze-drying were higher than those of nanoemulsions .The results implied elected methods effectiveness to prepare encapsulated Sweet violet extract, and obtained encapsulated extracts have the potential to be used in further pharmaceutical and food studies.

Keywords: *Flavonoid, Nanocapsule, phenol, Stability, Wall materials.*

1. INRODUCTION

Sweet violet (*Viola odorata* L.) is a perennial plant of Violaceae family. It grows in shady and humid areas which is distributed from Europe to Scandinavia, Southwest Asia, Turkey, Syria, Iraq, Iran, the Caucasus, and North Africa [1]. All aerial parts, seeds, and roots of this plant contain secondary metabolites, including flavonoids, anthocyanins, cyclotides, violin alkaloids, saponin, glycoside, methyl salicylate, and Viola flowers have medicinal properties, including anti-bacterial, antioxidant, anti-inflammatory, Antipyretics, treatment of liver disease, respiratory problems, treatment of cough, sore throat, hoarseness, laxative and sedative [2].

Herbal bioactive compounds' susceptibility to various factors and low solubility remains a great challenge for their

application in pharmaceutical industries and food [3]. Plant extracts were widely incorporated in nanoemulsions to improve the delivery of bioactive phytochemicals [4]. As an effective approach, Sweet violet extract's encapsulation was suggested; Yousefi et al. studied the preparation of chitosan-coated alginate microcapsules was shown with potential to be used in food products [3]. The synthesis of nanoparticles using the extracts of different plants can be more valuable than other biological syntheses, because due to the expansion of the distribution of plants, they can act as a source of several metabolites [5].

Nanocapsules were shown with important benefits, including the prevention of chemical reactions between the active substance and environment (ultraviolet light, oxygen, and moisture), increased storage time of active ingredient, ease of use by solidifying liquid core, and controlled release of materials [6,7]. Among many methods of encapsulating secondary metabolites, polymer-based nanoparticles are unique compared to other coatings due to their lower toxicity, better microencapsulation, and controlled release [8, 9]. Using gums as a polysaccharide-based polymer coating has increased. Using complex proteins and polysaccharides in the encapsulation of lipophilic materials, such as chia seed oil [10], mustard seed essential oil [11], Turmericoleoresin [12], palm oil and carotene [13], vitamin E [14], lutein [15] and lycopene [16].

Among many sub-micron particles, polymer-based nanoparticles are unique compared to other nanoparticle systems for better encapsulation, controlled release, and less toxicity. Choosing encapsulation method and materials depends on applying the encapsulated product [17].

Nanoemulsions are one of the methods that are known as miniemulsions, submicron emulsions and colloid. miniemulsions, submicron emulsions and colloids, which are stable [18]. Nanoemulsions are effective for the transfer of hydrophobic bioactive compounds by increasing the surface and bioavailability, the combination of lipophilic and hydrophilic drugs by increasing the absorption rate. These emulsions are placed in the oil phase and aqueous medium using emulsifiers such as Tween 80 [18]. In addition, processing parameters such as surfactant concentration, homogenization pressure, oil-to-water ratio, and the type of coating wall the values of hydrodynamic diameter and polydispersity of nano-emulsions are affected [19].

Gum Arabic is from the genus *Acacia* spp and Fabaceae family. This polysaccharide gum has as a coating wall its main chains contain galactose, and its subchains contain galactose sugars, rhamnose, arabinose, and glucuronic acid. Gum Arabic Charge is due to the existence of glucuronic acid in its structure and its viscosity changes with pH effect (Maximum viscosity at pH= 6-8). This gum has low viscosity, low molecular weight, and high solubility in water. It is used as an encapsulating agent due to its binding to proteins [20]. also The seed of Qodume shahri (*Lepidium perfoliatum* L.; Brassicaceae) is ellipsoid, small, and black. The grain's outer shell is swelled when placed in water and covered with mucilage (gum). This mucilage is used in the coating wall of nanoparticle compounds. The reason for extracting gum from Qodume seeds is its low price, high amount of mucilage, and high viscosity [21]. Nanocapsules are the technique of valuable complex materials and sensitive compounds inside a coating or wall with a size of 10 to 1000 nm [22]. The preparation of nanocapsules is done in two steps; first an emulsion of active compound such as medicinal compounds and fat-based flavor compounds are produced in a heavier solution (wall material), such as a polysaccharide or protein, and then dried [23]. Sweet violet has valuable medicinal active ingredients and has spread in many countries including the north of Iran, and is available to many researchers. This study was done to increase the effect, protection and stability of Sweet violet's bioactive

substances by encapsulation the Extracts and comparing two methods of nano-capsules preparation and two drying processes to select the most desirable method.

2. EXPERIMENTAL

2.1. Materials

Viola odorata L. flowers were collected from the Hezar Jerib area (Babolsar, Iran). A botanist identified the plant, and a voucher specimen was deposited at the herbarium of Research Institute of Medicinal plant, Sari University of Agricultural. *Lepidium perfoliatum* seeds, gum Arabic and sunflower oil were purchased from the local grocery (Babolsar, Iran). Sorbitan monooleate (span 80), Polysorbate 80 (Tween 80), and Gelatin (Bloom: 100-130) were from the Merck Company (Germany) with high purity.

2.2. Sweet Violet Extract Preparation

Extraction was done due to Yousefi et al.'s method with some modifications [3]. 5 gr of dried flowers in 100 mL of 80% ethanol Mix and placed in darkness at 25 °C for 24 hours. Then, the secondary metabolites were extracted by the ultrasonic device (Soltec Milano, Italy) at 50 °C for 15 minutes in three steps. Finally, the extract was filtered with filter paper. To thicken the extract, part of solvent was removed by a rotary evaporator (Vargha, Iran) for 20 minutes at 40 °C.

2.3. Extraction of Lepidium Perfoliatum Seed Gum

Seeds were soaked within deionized water at a pH of 7, and a ratio from 1 to 30 seeds at the temperature from 45 to 50 °C in a hot water bath (Fan Azma Gostar, Iran) was stirred for 20 minutes. After applying heat, the swollen seed and its shell were covered with mucilage, and the mucilage was extracted with a juicer (ParsKhazar, Iran). Finally, the seeds were discarded, and mucilage or seed gum was dried in an oven (Fan Azma Gostar, Iran) for 50 hours at 50 °C, and the powder was sieved with 18 mesh sifter [21].

2.4. Double Nanoemulsion

The encapsulation of secondary metabolites of Sweet violet (*viola odorata* L.) was done due to Shaddel et al.'s method with some modifications [24]. The ratio of the emulsion phase to aqueous phase was 60:30. The emulsion wall material (gum Arabic) was mixed in certain proportions to reach desired volume (0.05 g/ml) in deionized water, and a magnetic stirrer was used for 15 minutes at room temperature for better solubility of mixture. To complete the water absorption process, the material wall composition was kept in a refrigerator for 24 hours.

The ingredients ratio of primary emulsion (w/o), including liquid extract, span 80, and sunflower oil were 10:30:60. To prepare this emulsion, beginning Sweet violet extract was gently added to the combination of sunflower oil and Span 80, then placed on a shaker (Fan Azma Gostar, Iran) until the mixture became clear.

To produce a water-in-oil-in-water (w/o/w) emulsion, surfactant Tween 80 (ratio of twin and span 1:1) and gelatin solution (ratio of gelatin to gum Arabic 1:1) were added to primary emulsion. Then, the mixture was homogenized by homogenizer (Dragonlab, dlab D-500-1, China) in two steps at a speed of 12.700 rpm and each step for 4 minutes.

The gum Arabic solution was gently added to emulsion at 40 °C and pH 4 (adjusted with 0.1 M acetic acid) and mixed by a magnetic stirrer. Then, the applied temperature was slowly reduced to 10 °C. Finally, the double nanoemulsion formed was kept in the refrigerator for 24 hours and dried for 48 hours at -50 °C by freeze dryer device (Dena Vacum, FD-5003-BT, Iran).

2.5. Nanoemulsion

Lepidium perfoliatum seed gum was mixed with a concentration of 0.5% (weight-volume) in distilled water to form a wall coating, and a magnetic stirrer was

used for better dissolution at ambient temperature for half an hour. Finally, it was kept in the refrigerator for 24 hours to complete water absorption and bubble removal. To prepare the nanoemulsion, Sweet violet extract (2% w/w) was added dropwise to a mixture of wall solution (96 w/w) and tween 80 (2 w/w). Then, the emulsion was combined with a magnetic stirrer for half an hour and finally homogenized by a homogenizer device (Dragonlab, dlab D-500-1, China) in two stages at speeds of 12.700 rpm and 19700 rpm and each step for 5 minutes. To further reduce the particle size, an ultrasonic probe device (Bandeline, HD 3200, Germany) was used with 6 cycles of 30 seconds and a rest time of 15 seconds with an oscillation amplitude of 75%. The ice cubes were used around the emulsion container to prevent the emulsion temperature increase during homogenization and sonication [25].

2.6. Freeze-Drying

The obtained nanoemulsion and double nanoemulsion were stored at -20 °C for 24 hours and then in the freeze dryer (Dena Vacum , FD-5003-BT, Iran) for 48 hours at -50 °C and the pressure of 0.017 mP (mili pascal) dry and producing powders at -20 °C were conserved until sample analysis [24].

2.7. Spray-Drying

For drying nanoemulsions and double nanoemulsions from a spray dryer (Model B-191, BUCHI Mini) equipped with a 0.9 mm nozzle, drying power of 1.5 kg/h, inlet air temperature of 160 ± 10°C, outlet air temperature of 80 ± 10 °C, feed flow of 300-240 mL/h and compressor air pressure of 6-5 bar were used [26].

2.8. pH and Brix Measurements

Emulsions Brix was determined by a manual handheld refractometer (Kruss, HRB-10T, Germany), and their pH was measured by pH-meter (Sartorius, Germany).

2.9. Bulk Density

The weight (gr) of 5 mL of encapsulated powders was measured by balance. As a result, the powders' density was calculated due to nether formula [27].

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Bulk powered volume}} \times 100$$

2.10. Moisture Content

Nanocapsule powder (2 g) was poured into aluminum foil and placed in oven (Fan Azma Gostar, Iran) for 2-3 hours at 105 °C. After measuring the sample's dry weight due to the nether formula, the moisture content of sample was calculated [28].

2.11. Solubility

The powder (1 g) was added to 100 ml of distilled water and dissolved using a magnetic stirrer for 5 minutes at a speed of 385 rpm. It was centrifuged (Hettich, EBA 200, Germany) at 3000 rpm for 5 minutes. Finally, 25 mL of supernatant was poured into a petri dish and placed in an oven (Fan Azma Gostar, Iran) at 105 °C for 5 hours. Immediately after Taking out of the oven, the dry weight was measured, which amounted to the sample's weight, demonstrating the powder's weight dissolved in water [29].

$$S = \frac{M1 - M2}{0.25} \times 100$$

M1: Weight of petri dish and out of oven
M2: Petri dish empty weight
S: Solubility after 5 hours

2.12. Zeta Potential and Particle Size Measurement

Dynamic light scattering (DLS) (Malvern Zetasizer Nano, ZEN 3600, England) was used to measure PDI and particle size. The particle size was determined at a temperature of 25 °C with a 90 °C angle, and the polydispersity index (PDI) amount represents the distribution of particle size. Nanocapsules' zeta potential after dilution in double distilled water was measured using a zeta sizer.

2.13. Optical Microscope and Field Emission Scanning Electron Microscope (FESEM)

The shape, appearance, and integrity of nanoemulsion were analyzed by field emission scanning electron microscopy (FESEM) (Tescan, MIRA3, Czech Republic). The morphology of double nanoemulsion sample was observed before drying by an optical microscope.

2.14. Encapsulation Efficiency (EE)

An amount of 0.6 g of powder was added to 10 mL of alkaline water (PH =10.5) and dissolved with a magnetic stirrer for 30 minutes. It was centrifuged (Hettich, EBA 200, Germany) at 4000 rpm for 10 minutes. The supernatant was neutralized with concentrated acid, and the amount of phenol was determined by Folin Ciocalteu method. Encapsulation Efficiency was calculated by nether formula [30].

$$EE\% = \frac{Ce}{Ct} \times 100$$

Ce = amount Encapsulated phenolic compounds
Ct = initial amount of phenolic compounds
EE =Encapsulation Efficiency

2.15. DPPH Radical Scavenging Activity

DPPH radical scavenging activity of free and nanocapsulated Sweet violet extract was determined by the method of Wu et al. In this method, 1.5 mL of samples were mixed with 1.5 mL of DPPH radical solution at a concentration of 0.2 mM (dissolved in 95% ethanol) and placed in darkness on a shaker (Fan Azma Gostar, Iran) for 30 minutes. Finally, they were centrifuged (Hettich, EBA 200, Germany) at 4000 rpm for 10 minutes, and the absorbance of samples was recorded at 517 nm [31].

$$I\ \% = \frac{absc - abss}{absc} \times 100$$

absc: absorbance of control
abss: absorbance of sample
I(%): Scavenging Effect (%)

2.16. Emulsion Stability

Emulsion samples were transferred to 15 mL test tubes (internal diameter of 1.5 cm, height of 12 cm) immediately after formation. Their caps were sealed well and stored at room temperature for 10 days. During this period, the height of emulsions and separated layer were measured daily. Finally, the stability of emulsions was determined using nether formula [32].

$$CI = \frac{ht}{h0} \times 100$$

ht: height of apparently stable emulsion without any creaming
h0: Initial emulsion height
CI: creaming index

2.17. Emulsifying Ability

The emulsifying ability was investigated using centrifugation from Siarini et al. (2009) with some modifications. After preparing the emulsions, the samples were centrifuged (Hettich, EBA 200, Germany) at ambient temperature at 6000 rpm for 15 minutes. Considering the volume of separated phases, the emulsifying property index was measured [33].

$$ESI = \frac{f\ EV}{i\ EV} \times 100$$

f EV: final volume
i EV: Initial volume
ESI: Emulsifying ability

2.18. FTIR Analysis

This method was used to determine organic compounds and their functional groups. This device qualitatively determined the compounds and their interactions by determining the absorption intensity from peaks. In the analysis of sample (Sweet violet extract, nanocapsules containing the extract and coating wall compounds) by Fourier Transform Infrared Spectroscopy (FTIR) device (Cary 630, Agilent Technologies, USA) scanned in the 600-4000 cm^{-1}wave range.

2.19. Statistical Analysis

Data analysis was done using software SPSS ver 24 in a completely randomized design. The means of three replications

were compared using Duncan's test at 95% level.

3. RESULTS AND DISCUSSION

3.1. pH and Brix Measurements

The measured brix values were observed for 15% double nanoemulsion and 1% nanoemulsion (Table 1).

Table 1. *Physicochemical properties of the Double nanoemulsion and nanoemulsion of Sweet violet extract.*

Samples	pH	Brix (%)	Zeta potential (mV)	particle size (nm)	PDI	DPPH (%)	emulsion stability (%)	emulsifying ability (%)
1	3.34	15	-6.2	798.3	0.443	136.67	79.66	79.66
2	6.2	1	-19.3	306.5	0.478	65	97.75	99.84

Sample 1: *Double nanoemulsion with gum arabic and gelatin before drying.* ***Sample 2:*** *Nanoemulsion with Lepidium perfoliatum L. gum before drying.*

Each emulsion has the most stability at its optimum pH due to the increase in zeta potential at that pH. The amount of zeta potential always changes as pH changes [34]. Considering Table 1, the pH of double nanoemulsion is 3.34 and nanoemulsion is 6.2. Also, the type of surfactant used in nanoparticles is effective on the pH value [35].

3.2. Bulk density

The bulk density depends on shape, size, and surface properties of powder particles, which smooth and uniform powders have a higher volumetric density [36]. The free energy density of the surface is one of the most important physical properties that determine the nature of the surface effect. For nanoparticles, a strong size effect of the surface energy density is shown when the particle diameter is less than a few nanometers. As a result, with the increase in the diameter of nanoparticles, the surface energy density decreases [37].

Comparing the average density of samples, double nanoemulsion with gum Arabic coating (0.31 g/mL) has a significant difference with nanoemulsion with *Lepidium perfoliatum* L. coating by freeze dryer method (0.122 g/ mL) and spray dryer method (0.142 g/mL) (Table 2). The numerical value of emulsion density with gum Arabic coating is more than *Lepidiumperfoliatum* L. gum. As a result, in the same mass of samples, emulsions with gum arabic have less porosity and less filler

The amount of moisture affects the amount of density which the higher the moisture content, the higher the mass and density.

Table 2. *Physicochemical properties of the Double nanoemulsion with gum arabic and gelatin and Nanoemulsion with Lepidium perfoliatum L. gum.*

	Bulk density (g/cm3)	Moisture content (%)	Solubility (%)	EE (%)
1	0.31	5.2	24.52	85.82
2	0.122	1.83	25.03	56.31
3	0.142	1.83	25.36	69.94

Sample 1: *Double nanoemulsion with gum arabic and gelatin Drying by freeze dryer (GA:GE/FD).* ***Sample 2:*** *Nanoemulsion with Lepidium perfoliatum L. gum Drying by freeze dryer (LPSG/FD).* ***Sample 3:*** *Nanoemulsion with with Lepidium perfoliatum L. gum Drying by spray dryer (LPSG/SD).*

As the moisture increases, the particles tend to stick together which reduces the space among the particles [38,39].Less interstitial space reduces the oxygen available for decomposition reactions which destruct the rapid degradation of coating material [39].

3.3. Moisture Content

Considering Table 1, the emulsion moisture content with gum Arabic coating (5.2%) was higher than the moisture content of emulsion with *Lepidium perfoliatum* L. gum coating (1.83%). Gum arabic has a heteropolysaccharide complex with a branched structure which has hydrophilic groups. It binds to water molecules and prevents them from leaving [32]. Hence, the type of wall material affects the final moisture content of powders. In Chranioti and Tzia's study (2014), the moisture content of the microcapsules of secondary metabolites fennel (*Foeniculum vulgare*) by gum Arabic was 4.56%which is due to the measured results [40].

3.4. Solubility

Considering the obtained results (Table 2) the percentage of double nanoemulsion solubility is less than nanoemulsion which there is no significant difference among samples. Due to Ghasemi et al.'s theory (2017), the solubility increases by increasing pH. Also, the solubility is greatly influenced by the use of hydrophilic polymers as surfactants and carriers and reducing the particle size, which increases solubility [41]. The nanocapsules' pH was measured by gum Arabic 3.34 and *Lepidium perfoliatum* L. gum 6.2; so, the solubility of gum Arabic is less than *Lepidium perfoliatum* L. gum. A strong bond formed among wall materials became stronger as pH decreased; so, their solubility in water decreased. At pH 3.34, nanocapsules with gum Arabic coating have a larger particle size (798.3 nm) than *Lepidium perfoliatum* L. gum (306.5 nm); so, larger particles need more time to dissolve in water. *Lepidium perfoliatum* L. gum has a low solubility in water which is an important factor in the solubility percentage of *Lepidium perfoliatum* L. gum nanocapsules [29].

3.5. Zeta Potential

Zeta potential is a good method to predict colloidal systems stability [42]. In this study, the zeta potential of secondary metabolites' nanocapsules was measured by gum arabic and gelatin -6.2 mV and *Lepidium perfoliatum* L. gum -19.3 mV. The electric charge in suspensions depends on the polymer's chemical properties, the pH of environment and stabilizer type. The cause of positive charges in gelatin may be related to arginine and lysine's residual presence in arginin. Among proteins, gelatin is a suitable selection for composite wall production due to its amphoteric nature. Gum Arabic also contains magnesium, potassium, and calcium salts which cause a negative charge after the gum Arabic dissolved in water. This gum creates a strong interaction with a positive electric charge [43]. In general, the closer the zeta potential is to the range of 30 mV at positive charges and -30 mV at negative charges, the more stable it is [44]. As a result, by positive and negative charge interaction in double nanoemulsion wall with a zeta potential of -6.2 mV, it has less stability than *Lepidium perfoliatum* L. gum zeta potential of -19.3 mV. Considering the analyzes, the PDI value in both emulsions was less than 0.5, Encapsulating of Sweet violet extract in both methods has a homogeneous particle size distribution and polydispers (Figure 1).

3.6. Particle Size

Due to Figure 1, nanoemulsion with *Lepidium perfoliatum* L. gum was measured 306.5 nm and double nanoemulsion with gum Arabic coating 798.3 nm. *Lepidium perfoliatum* L. seed gum contains 88.23% carbohydrates and 4.6% protein which in water causes high adsorption capacity, low solubility, stability, and increased viscosity of

environment [21]. Therefore, *Lepidium perfoliatum* L. has a high viscosity causes more resistance to Particle motion, prevents them from joining which leads to smaller particle sizes [45]. Shadel et al. (2018) used the modified method to form a double nanoemulsion with gum arabic and gelatin. The particle size analysis results from 26.57 to 36.36 micrometers, the studies are not conformity with their results.

The Nanocapsule production method has a significant effect on particle size and distribution. In addition to changing nanoemulsion formation method, the differences in emulsifying properties of these two gums, such as surface activity properties, their adsorption rate at the drop surface, and Intermolecular interaction are related [24, 46].

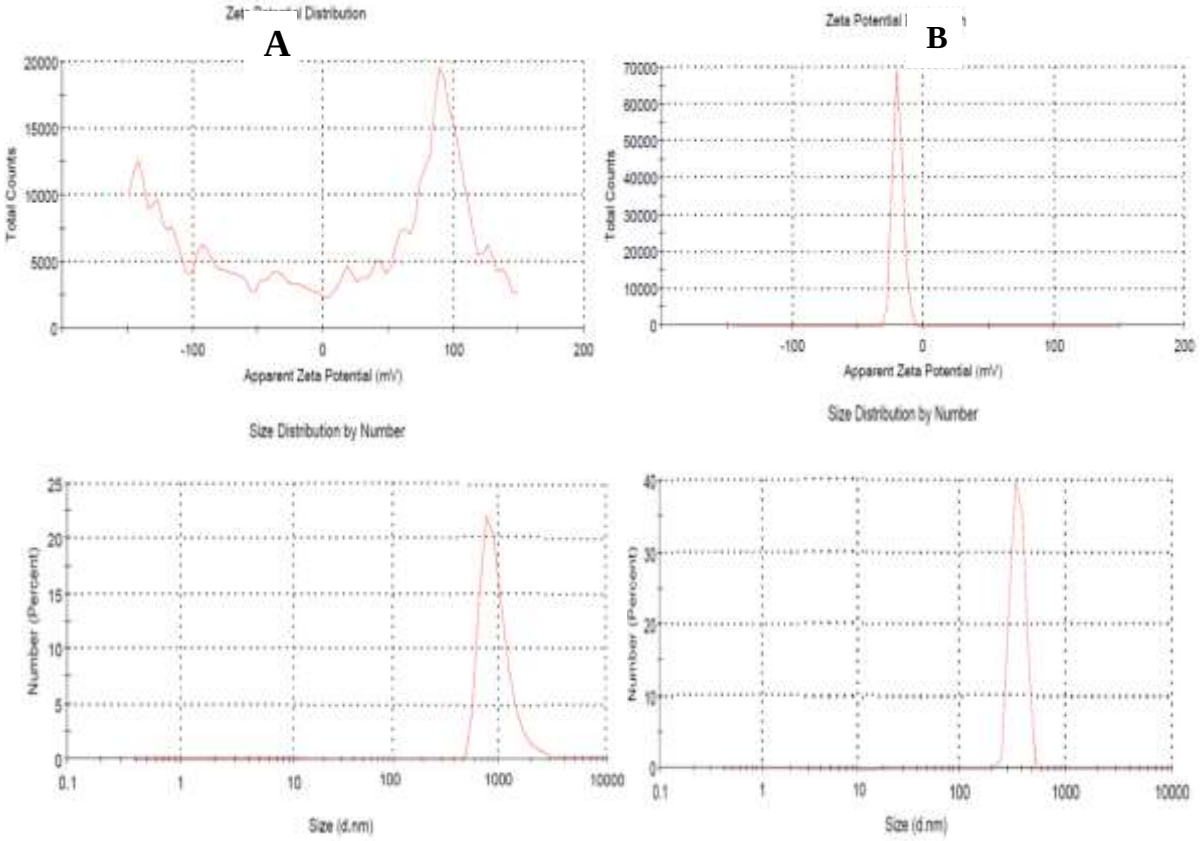
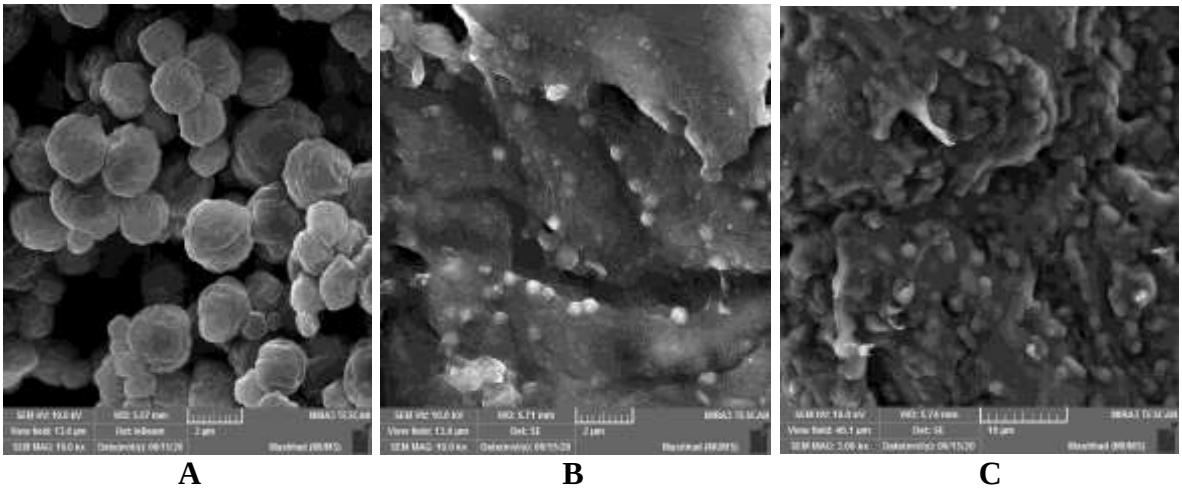


Figure 1*. zeta potential and Particle size of (A) Double nanoemulsion, (B) Nano emulsion.*



A **B** **C**

Figure 2. *FESEM image of nanocapsules produced by freeze drying and spray drying with difrent wall material. (A) spray drying with Lepidium perfoliatum L. seed gum (LPSG/SD) (B)*

freeze drying with Lepidium perfoliatum L. seed gum (LPSG/FD) (C) freeze drying with gum arabic and gelatin (GA:GE/FD).

3.7. Optical Microscope and Field Emission Scanning Electron Microscope (FESEM)

The nanocapsules were dried by spray and freeze dryer, due to the high percentage of oil in the double nanoemulsion sample, spray dryer method was not suitable. Therefore, the spray drying method was used for nanoemulsion with *Lepidium perfoliatum* L. gum coating (Figure 2A and B). For double nanoemulsions coated with gum arabic before drying by optica microscopic, their morphological structure was observed in micro range (Figure 3). DLS peaks show larger sizes than FESEM results. In DLS data, the size of large particles can be due to the interaction of different forces in ionic conditions and hydrodynamic environment [47].

Considering encapsulation results by spray dryer (spray dryer), the particle size was in micro range, while it was observed by DLS analysis in nano range; so, drying method is effective in particle size. There are several reasons for increasing the sample size after drying.

The stability reduces emulsions during spray drying, the spray dryer nozzle's size, and the type of wall material. Due to Figure (Figure 2A), it is morphologically regular, spherical with Low shrinkage, without cracks, fractures, and depression. The formation of shrinkage and depression on the capsules' surface is due to the rapid formation of the wall and swelling inside the particle due to increased vapor pressure and increased particle temperature [48]. Considering Figure (Figure 2B), using freeze dryer, secondary metabolites of Sweet violet with *Lepidium perfoliatum* L. gum coating, compared to spray dryer, its morphological structure is not well known.

3.8. Encapsulation Efficiency

Various factors including the properties of capsule wall material, the ratio of core material to wall, drying conditions and emulsion formation conditions. Effect of the encapsulation efficiency [48].

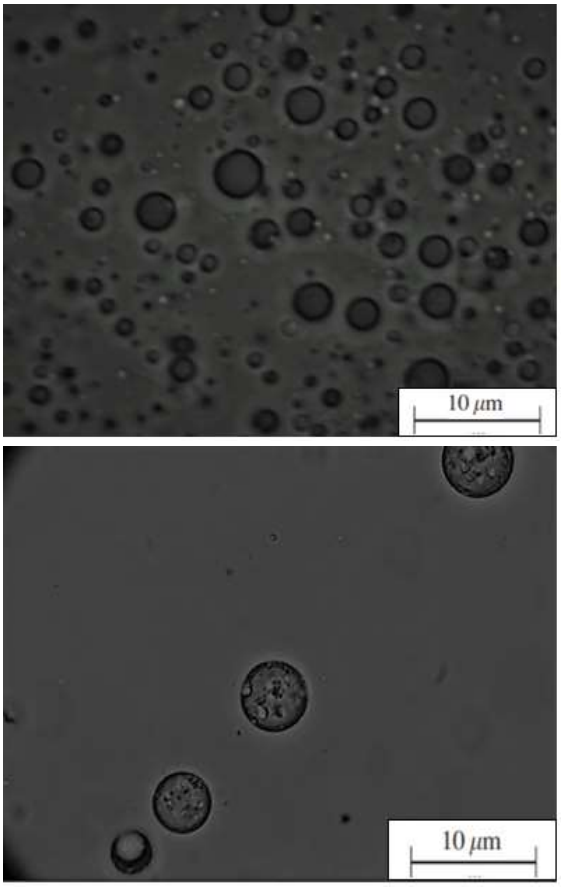


Figure 3. *Optical micrographs of double nanoemulsion Sweet violet extract before freeze drying with gum arabic and gelatin wall (GA:GE)*

Due to Figure 4, the amount of encapsulation efficiency of the secondary metabolites of Sweet violet was measured by gum Arabic and *Lepidium perfoliatum* L. by freeze dryer and spray dryer methods was 85.82%, 56.31%, and 69.94% , respectively. Gum Arabic had the highest encapsulation efficiency and a significant difference was observed among samples.

Oil encapsulation efficiency (EE) is an important factor for nanoformulation. In a research, the encapsulation efficiency was positively correlated with the amount of garlic oil, which increased with the addition of garlic oil concentration [49].

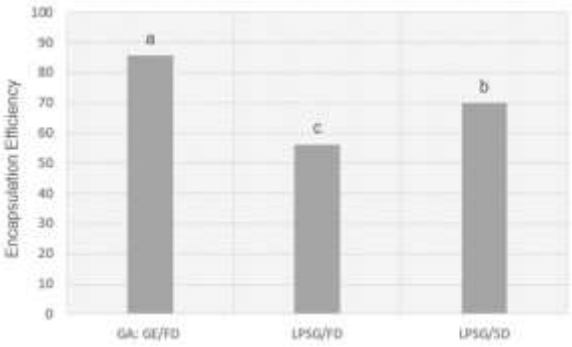


Figure 4*. Encapsulation Efficiency of nanocapsules produced by freeze drying and spray drying with different wall material. (A) spray drying with Lepidium perfoliatum L. gum (LPSG/SD) (B) freeze drying with Lepidium perfoliatum L. gum (LPSG/FD) (C) freeze drying with gum Arabic and gelatin (GA:GE/FD)*

Therefore, in our study, the appropriate ratio of oil in the double nanoemulsion of gum arabic increased the microencapsulation efficiency.The reason for decrease in the encapsulation efficiency of mucilage coating compared to gum Arabic can be related to the sensitivity of phenolic compounds to various conditions such as the presence of heat, light, and oxygen during process which led to a decrease in the efficiency of active compounds [50]. The hydrophilic nature of extract can affect this efficiency. During the encapsulating process, part of extract due to its hydrophilic nature, enters the aqueous phase and reduces efficiency [51].

3.9. DPPH Radical Scavenging Activity

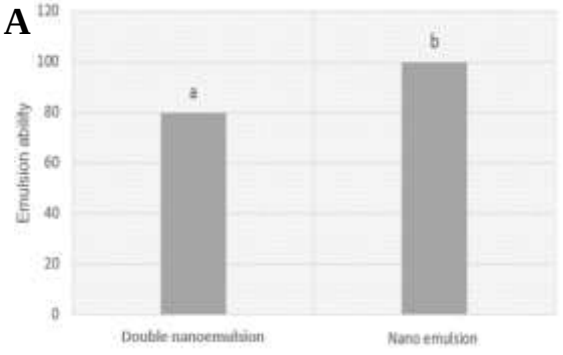
The highest antioxidant activity was observed in double nanoemulsion with gum Arabic (136.67%) which significantly differs from the extract and nanoemulsion with *Lepidium perfoliatum* L. gum (Figure 5A). Due to the obtained results, nanocapsules' antioxidant activity with gum Arabic coating is higher than free extract, and *Lepidium perfoliatum* L. gum is less than free extract. Water-in-oil emulsions have more free radical scavenging properties than water-in-oil nanoemulsions due to phenolic compounds in oil phase. Outer phase of oil-in-water nanoemulsions is distilled water and free of any phenolic substances. Therefore, the higher antioxidant activity of gum Arabic emulsion than free extract can be due to phenolic compounds. The interaction between antioxidants and phenolic compounds with coating wall compounds of *Lepidium perfoliatum* L. emulsion can reduce the antioxidant activity of nanocapsules [52].

3.10. Stability Index and Emulsifying Ability

Considering the obtained creaming index results (Table 2), nanoemulsions with a coating of *Lepidium perfoliatum* L. gum (97.75%) are more stable than gum Arabic (45.38).

Due to the high molecular weight of hydrocolloids, by adding them to the emulsions, they reduce the droplets' movement and increase the viscosity of the emulsion. The amount of particles efficiency with each other is reduced.

Increasing the stability index of emulsion with *Lepidium perfoliatum* L. gum compared to gum Arabic is due to the higher viscosity of *Lepidium perfoliatum* L. gum. Considering mentioned results, the zeta potential of nanoemulsion was more than double nanoemulsion which proves the reason for more stability of this emulsion [53]. In the results obtained from the emulsifying ability, its amount was more than Arab gum (79.66) with coating of *Lepidium perfoliatum* L. gum (99.84); so, these results show more stability of nanoemulsion (Figure 5B).Therefore, viscosity plays an important role in emulsion stability.



B

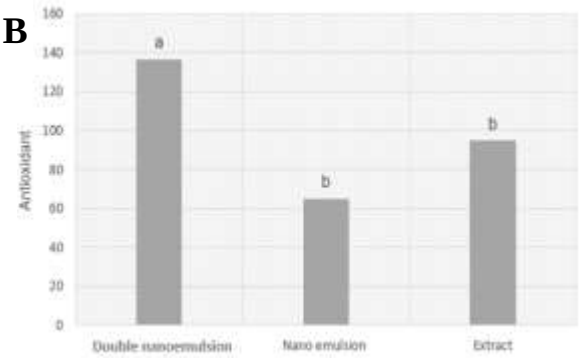


Figure 5. *Antioxidant (A) and Emulsion ability (B) of emulsion*

3.11. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy analysis was performed to identify the functional groups also the possible interactions of coating wall compounds in the formation of sweet violet extract nanocomplex. The peaks obtained from this experiment were shown due to Figure 6. Figure A and B's peaks are the characteristics of the functional group of wall compounds (gum Arabic and gelatin) and double nanoemulsion and sweet violet extract. Functional groups of wall compounds include O-H, N-H, S=O, C-F, CO, C=C, C-H, C-Br, and C-Cl. Double nanoemulsion complex functional groups such as O-H, NH, C=C, C-F are similar to wall compounds, other double nanoemulsion functional groups include S=O of sulfoxide, sulfone, sulfonic acid, sulfonamide, sulfonate, sulfate and sulfonyl chloride, C-O group in alkyl aryl ether, vinyl ether and ester, C-N group in aromatic amine, C=O group in esterster and C-H group in an alkene. The sweet violet plant extracts due to Figure 6C has O-H, NH, CF, C=C, C-Br, and C-Cl functional group similar to emulsion coating wall compounds and S=O group of sulfate, sulfonyl chloride, sulfonate compounds and sulfonamide. Considering Figure 6D, at a wavelength of 3318.45 cm^{-1}, O-H functional group of the carboxylic acid compound of extract disappeared. The extract's adsorption with a wavelength of 2923.55 cm^{-1} was not observed in double nanoemulsion complex; so, the disappearance of these peaks proves nanocapsules formation [54, 55, 56]. Therefore, nanocapsule complex formation was done using coating wall compounds (gelatin and gum Arabic). As a result of the interaction of gelatin and gum Arabic with positive and negative charges due to carboxylic acid and amines functional group, a new functional group of amides was formed at the wavelength of 1457.10 cm^{-1} which indicates the interaction of polymers and the production of double nanoemulsion complexes [24].

Considering the nanoemulsion of sweet violet extract due to Figure 6E, peaks are the characteristics of the functional group of wall composition (*Lepidium perfoliatum* L. gum) and nanoemulsion of freezer and spray dryer. The functional group of *Lepidium perfoliatum* L. gum is O-H, N-H, C-H, C=O, S=O, and C-F. In nanoemulsions complex (freeze dryer and spray dryer), N-H, C-F, C-H groups similar to wall compounds (*Lepidium perfoliatum* L. gum) and C=O group of aldehyde, C=C group of alkene, S=O group of sulfonic acid, sulfate, sulfone, sulfonamide, sulfonate, sulfoxide, O-H group of phenol, carboxylic acid and alcohol, C-N group of amine, C-O group of alkylaryl ether, aromatic ester, aliphatic ether and C-Cl group of halo compound. Considering the results (Figure 6F), a wavelength of 3408 cm^{-1}, the O-H functional group of carboxylic acid compounds and wavelength of 2920.95 cm^{-1}, N-H group from amine compounds of extract disappear which proves the encapsulation of extract. The new wavelengths created include 2859.61 cm^{-1}, 1734.88 cm^{-1}, 1348-96 cm^{-1}, and 1247.60 cm^{-1}, the functional groups of *Lepidium perfoliatum* L. gum compounds in these frequencies, prove nanoemulsion formation (freeze-drying). In the nanoemulsion (spray drying), OH functional groups from carboxylic acid at 3475.753 cm^{-1}, S=O group from sulfonate, and sulfoxide at 1419.104 cm^{-1}, and the C=C group from alkene were removed, as

seen in sweet violet extract. So, the change of peaks indicates the production of nanocapsule complex (Figure 6G) [57].

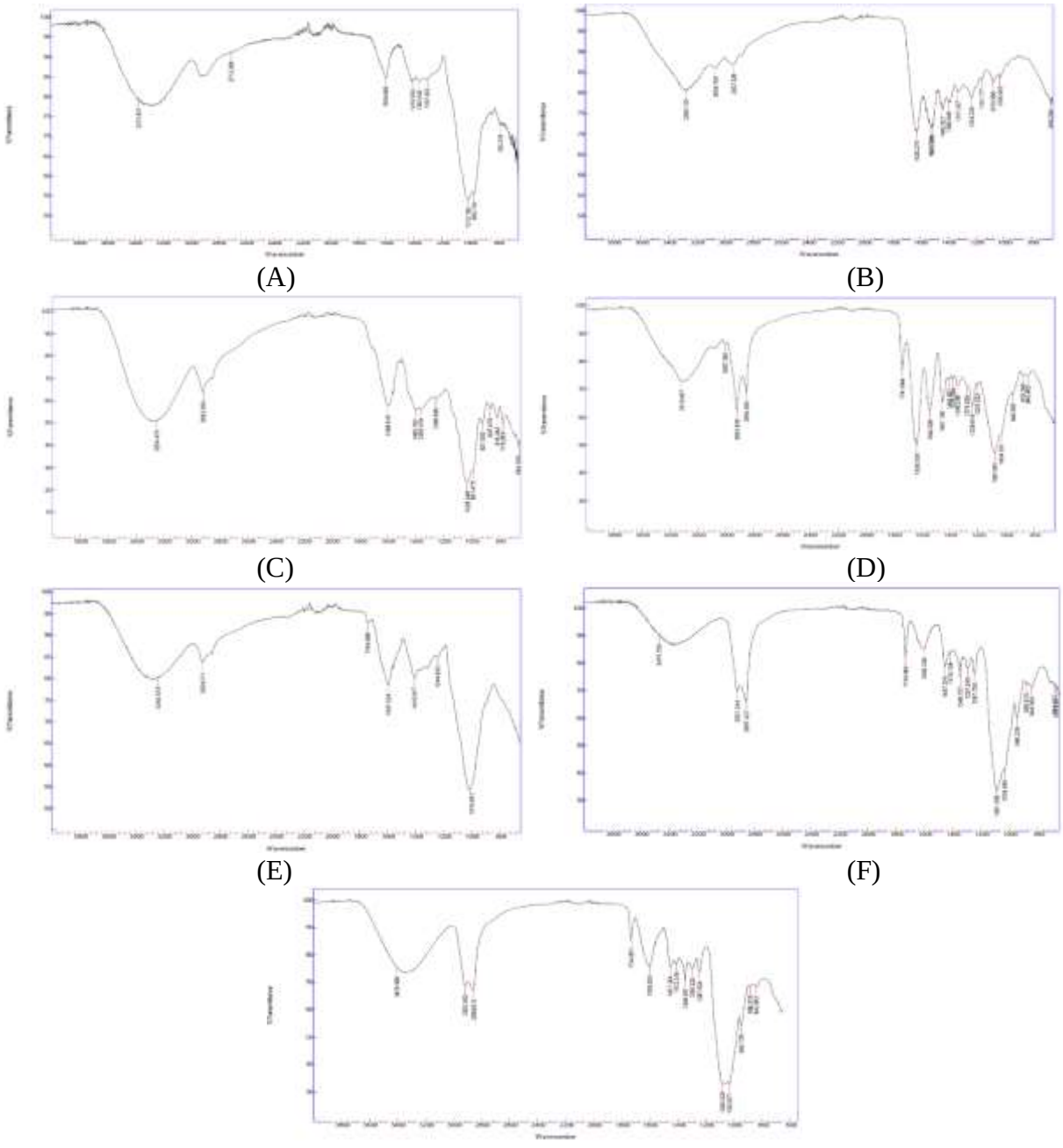


Figure 6. *FTIR spectra of (A) gum arabic, (B) Gelatin, (C) Extract, (D) Double nanoemulsion (freeze drying), (E) Lepidium perfoliatum L. gum, (F) Nano emulsion (spray drying), (G) Nano emulsion (freeze drying).*

4. CONCLUSIONS

In this study, the Bioactive compounds of *viola odorata* L. plant were encapsulated using *Lepidium perfoliatum* L. and gums Arabic and protein biopolymer (gelatin). To encapsulate, two methods of double nanoemulsion and nanoemulsion were used which finally dried by a freeze dryer and spray dryer. As a result, the highest encapsulation efficiency of 85.82% and more than antioxidant activity of 136.67% was in double nanoemulsion with gum

Arabic-gelatin coating to the higher zeta potential of nanoemulsion with *Lepidium perfoliatum* L. gum coating, they are more stable. The lower zeta potential in double nanoemulsion is due to gum Arabic interaction with gelatin. Therefore, the optimal stability of this emulsion, the amount of emulsifying ability, is desirable. Considering the biological activity of aromatic violet extract and its applicability in the food industry, both methods can be further studied to increase the stability and encapsulation efficiency.

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