



**Scientific Research**

**Extraction of nanoparticles of Avondol plant (*Smyrniun cordifolium* Boiss) and its use in the preparation of alfalfa seed mucilage biocomposite and milk thistle seed oil and investigation of its physicochemical properties.**

Fatemeh Khakpour <sup>1\*</sup>, Sajad Pirsā <sup>2</sup>

- 1- Master's student, Department of Food Science and Industry, Faculty of Agriculture, Urmia University, Urmia, Iran.
- 2- Department of Food Science and Industry, Faculty of Agriculture, Urmia University, Urmia, Iran

**ARTICLE INFO**

**ABSTRACT**

**Article History:**

Received: 2023/10/4  
Accepted: 2023/12/12

**Keywords:**

Edible film,  
mucilage,  
Avondol nanoparticles,  
milk thistle essential oil

**DOI: 10.22034/FSCT.21.147.114.**

\*Corresponding Author E-Mail:  
[sevdakhakpour1@gmail.com](mailto:sevdakhakpour1@gmail.com)

Study background: adding *Smyrniun cordifolium* nanoparticles and milk thistle essential oil can improve the physicochemical properties of films based on alfalfa seed mucilage. Purpose: The purpose of this study was to prepare edible films from alfalfa seed mucilage with *Smyrniun cordifolium* nanoparticles (0, 2, 4%) and milk thistle essential oil (0, 1, 2%). Methodology: Edible films based on alfalfa seed mucilage were prepared and *Smyrniun cordifolium* nanoparticles (0, 2, 4%) and milk thistle essential oil (0, 1, 2%) were added to it. The physicochemical properties of the prepared films were investigated. Results: According to the obtained results, increasing the amount of *Smyrniun cordifolium* nanoparticles and milk thistle essential oil in the film decreased the mechanical properties of the films. The results of antimicrobial activity showed that the addition of *Smyrniun cordifolium* nanoparticles increased the activity of the film against *Staphylococcus aureus*. The dynamic light scattering (DLS) results confirmed the *Smyrniun cordifolium* nanoparticles. Fourier transform infrared (FTIR) results confirmed the physical presence of *Smyrniun cordifolium* nanoparticles in the polymer matrix. The increase of *Smyrniun cordifolium* nanoparticles and milk thistle essential oil in the films could delay the thermal decomposition of alfalfa seed mucilage and increase the thermal stability of the mucilage film. Final conclusion: Adding *Smyrniun cordifolium* nanoparticles and milk thistle essential oil to edible films based on alfalfa seed mucilage improved the antimicrobial, Fourier infrared (FTIR) properties of the films, and also weakened the mechanical properties.

## 1. Introduction

Due to the increase in the production and consumption of petroleum polymers and plastics in human daily life, diseases caused by food poisoning have become a great threat to human health. Since a major part of the packaging industry is plastic, the packaging industry can be linked to petroleum products [1]. Therefore, the ever-increasing growth of the population, the pollution caused by the packaging materials of petroleum derivatives, and the problems caused by the various methods of disposal of these materials, including burning, burial and recycling, have drawn more attention to biopolymers and biopackaging. Biodegradable films and coatings are suitable alternatives to synthetic films in the packaging industry due to their compatibility with the environment and low dependence on non-renewable resources, and have attracted the attention of many researchers. [2-3]. Synthetic plastics used for packaging. Different types of food cause serious environmental problems. The environmental effects of using plastic in the food industry have encouraged the packaging industry to produce packaging from renewable materials [4-5]. Biodegradable packaging protects food products against mechanical, physical and chemical damage and prevents their quality from deteriorating. It can also prevent microbial activity as an antimicrobial carrier in the form of antimicrobial packaging and increase the shelf life of food products [6-7]. In recent years, many studies have been done in the field of replacing synthetic polymers with biodegradable polymers, especially for food packaging. Most of the disadvantages and problems of synthetic polymers due to their biodegradability in nature have not been discussed for biopolymers, and this has caused many researchers to use all types of biopolymers in the study of food packaging to make biological packaging. It is considered decomposable [8-9]. The main ingredients of the biopolymer film are proteins, fats and polysaccharides. These materials can be used alone or in combination. The physical and chemical properties of biopolymers have a

great influence on the properties of the films made from them. Film constituents can be hydrophobic or hydrophilic [10]. Among the polysaccharide compounds that are used to form edible films are mucilages and granular gums. Mucilages are biopolymer compounds that are used in the production of various compounds, both medicinal and food, and in this regard, plant mucilages are preferred because they are non-toxic, have a low price, and are abundant [11]. It has been used to prepare edible film, mucilage is alfalfa seed, which is used in the present study. Mucilage can occur directly as a jelly-like structure in vegetative parts of plants (fruits, leaves, flowers, roots or stems) as well as in seeds after water treatment. From a chemical point of view, mucilages are large molecules that mainly contain carbohydrates and uronic acids, as well as glycoproteins and other bioactive compounds [12]. Mucilages have a wide range of applications: in food and nutraceuticals as structuring, gelling, texture, and film forming, in pharmaceuticals as binders and disintegrants for drug delivery systems, and in cosmetics as stabilizers. They have also attracted a lot of interest in the textile and paper industries, and they can be used in the production of paints. Alfalfa (*Medicago sativa* L.) is a herbaceous and perennial forage legume. All over the world, alfalfa is known for its high nutritional value as fodder and animal feed, as well as for its sustainable characteristics, excellent adaptation to extreme weather conditions and environmental flexibility, for example, alfalfa contributes to soil conservation, stabilization Nitrogen helps reduce soil pollutants, air pollutants, carbon dioxide deposition, etc. [13]. A protein material with the composition of essential amino acids similar to that of soy protein concentrate and 17-27% of soluble and insoluble dietary fibers [14] In addition, a series of micronutrients such as carotenoids, tocopherol, polyphenols, saponins B-complex vitamins and minerals have been identified [15]. Milk thistle has been used for medicinal purposes for more than 2000 years, mostly to treat liver diseases (cirrhosis and hepatitis) and

also to protect the liver from toxic substances. The therapeutic effects of St. John's wort are closely related to the presence of flavonoids. A complex called silymarin, consisting of a mixture of silybin A and B, isosilybin A and B, silychristin, and silydianin [16] is also made from the whole plant for medicinal purposes for treatment. It is used for kidney, spleen, liver and gall bladder diseases [17]. Silymarin also showed good antioxidant, anti-inflammatory and anti-fibrotic compounds. It was found to stimulate protein biosynthesis, increase lactation, and possess immunomodulatory activity. In addition, silymarin inhibits cell growth, DNA synthesis, and other mitogenic signals in human prostate, breast, and cervical cancers. [18]. *Smyrnum cordifolium* Boiss is a medicinal plant belonging to the Apiaceae family and among the native plants of Iran, which grows well in the heights and slopes of the Zagros mountain range in the western regions of Iran [19]. Sesquiterpenes, monoterpenes and flavonoids are among the main compounds found in the essential oil of this plant species [20]. The ball mill method is a common method for producing fine powder in many industries [21]. and is a tool of chemical mechanics and an expanding branch of chemistry. Ball milling of natural materials can bring their application potential to a new level [22]. In addition to reducing the particle size to the nanoscale [23], high-energy ball milling can also change the surface structure due to the mechanochemical effect. Ball milling can have severe effects on plant cell walls. The mode of work, as well as the materials of the milling jar and balls, and the type and amount of raw materials affect the result of milling [24]. Among the energetic ball mills, the planetary mill is a versatile and simple mechanical device for efficient grinding. Recently, planetary mills are used to prepare fine powders from all kinds of plant, mineral, chemical, ore, alloy, glass and ceramic materials. A planetary mill can convert particles into fine powders based on mechanical energy transfer, or impact and friction forces through high-hardness conductive ball balls, and is often used in laboratories and industry to produce materials with small particle sizes. [25]. Ball milling involves the movement of

balls that impart kinetic energy to the material being milled, breaking chemical bonds and creating fresh surfaces by breaking up the material particles. In order to improve the properties that the raw material lacks, such as solubility, dispersibility, surface effects, and chemical reactivity, ultrafine grinding technology has been developed [26]. The advantages of ball mill with high energy can be mentioned as flexibility, simplicity, ease of handling, capacity for large production periods. *Smyrnum cordifolium* Boiss was converted into nano particles and using DLS, the average size of the extracted nano particles was taken, and then an edible film was prepared by mixing mucilage of alfalfa seeds with nanoparticles of avondol plant and milk thistle seed oil. According to the investigations, there has been no research on the effect of Avondol nanoparticles and milk thistle essential oil on edible films prepared from alfalfa seed mucilage. The purpose of this research is to investigate the effect of Avondol nanoparticles and milk thistle essential oil in different concentrations on the physicochemical properties of edible films prepared based on alfalfa seed mucilage.

## 2- Materials and methods

### 2-1-Materials

Mucilage using alfalfa seeds, essential oil using milk thistle and nanoparticles using extracted avondol were used. Sodium nitrate, 99% methanol, glycerol, silica gel and other chemicals and solutions were obtained from Merck (Germany) and Sigma-Aldridge (USA) and were used without further purification.

### 2-2- The method of preparing mucilage from alfalfa seeds

Alfalfa seeds were mixed with distilled water at a ratio of 1 to 20 and first exposed to ultrasound at a temperature of 20°C and then placed on a magnetic stirrer at a temperature of 50°C for 2 hours. Then the entire contents were passed through a fabric filter. The remaining grains on the fabric filter were mixed again with a smaller proportion of distilled water and after stirring for 1 hour, it was passed through the fabric filter. Then the mixture obtained from the previous step was centrifuged for 10 minutes at a speed of 4000 rpm. The mucilage obtained

from the previous step was dried using an oven at a temperature of 40°C and stored in a zipped bag [27].

### 2-3- Preparation of essential oil from milk thistle

Its essential oil was extracted from milk thistle seeds by distillation with water using a colonger machine. In this way, the milled milk thistle seeds were poured into a balloon and about three times the amount of distilled water was added. By closing the connections of the balloon and the refrigerant and showing the flow of cold water related to the refrigerant and heating the balloon, degreasing was done and water was extracted with anhydrous sodium sulfate [28].

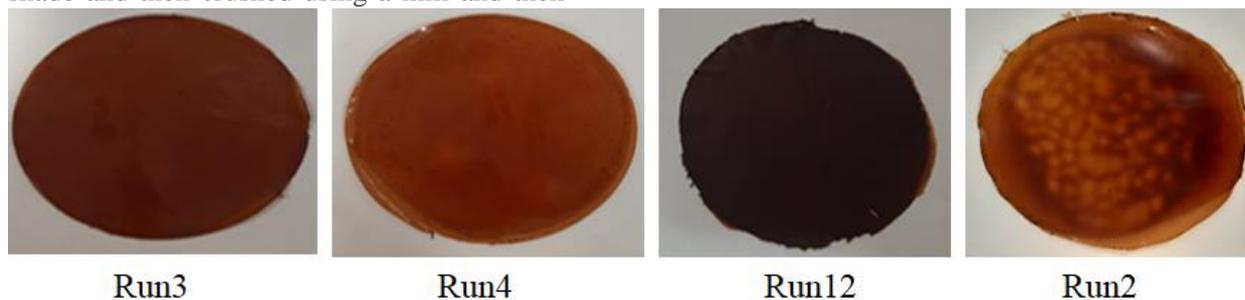
### 2-4- Nanoparticle extraction from Avondol plant

First, the stem and leaves of the Avondol plant were collected from the heights of the mountains and then dried for four days in the shade and then crushed using a mill and then

sieved using a micrometer sieve and then placed in a planetary mill for six hours. It was placed at a speed of 6000 rpm. After every hour and ten minutes, the rotation of the planetary mill stopped. And finally, the powdered nanoparticles are stored in a black container.

### 2-5- Production of films

First, 2 grams of alfalfa seed mucilage was poured into 80 ml of distilled water and stirred using a magnetic stirrer at a temperature of 70 degrees Celsius and a speed of 500 rpm. And different percentages of Avondol nanoparticles (0, 2, 4%) and milk thistle essential oil (0, 1, 2%) were dissolved in 20 ml of distilled water and added to alfalfa seed mucilage solution. After adding 15% glycerol to the solution, pH was adjusted to 7 using NaOH solution. The solution was poured into the Falcon and centrifuged. The resulting supernatant solution was poured into the plate and after 48 hours, the films were dried at room temperature, then the dried films were stored in zipped bags [29].



**Fig 1:** An image of the prepared films

### 2-6- Characteristics of films

#### 2-6-1- Dynamic Light Scattering (DLS)<sup>1</sup>

Particle size or zeta potential The mean diameter, size distribution or zeta potential of the samples were tested using a particle size analyzer, 3000 Zetasizer Nano-ZS Malvern. A sample of nanopowder was dispersed in double-distilled water (as a solvent) at a temperature of 25 °C. The sample was exposed to photon beams, which caused the particle to react. The scattering of the particle beam caused fluctuations in the intensity distribution, which were collected at the corner of interest

and then detected by a sensitive detector. The sample suspension was only sonicated for 60 minutes [30].

#### 2-6-2- Measurement of color characteristics

Color parameters (Hunter L, a, b) were measured using the Hunter lab system (Colorimeter Minolte CR-400). In order to calibrate the device, a standard white screen was used to measure the color of the films. The factors determined in this device include L or film brightness (0 to 100), a green-red (-80 to 100) and b blue-yellow (-80 to 70) [29].

#### 2-6-3- Measuring the mechanical properties of the film

1- Dynamic light scattering

Tensile tests were performed with a tissue analysis device. The force of 200 newtons, the distance between the two jaws of the machine was 40 mm and the speed of movement was 50 mm/min. Then the tensile properties of the films, including tensile strength (TS)<sup>2</sup> and percentage of elongation at the point of rupture (%E) were evaluated according to the ASTM D882-12 standard method [29].

#### 2-6-4-Inspection of antimicrobial properties

In the agar diffusion method, films with a diameter of 15 mm were cut into discs and placed on Mueller Hilton agar culture medium and plates containing *Escherichia coli* and *Staphylococcus aureus*. Then the plates were kept in a greenhouse at a temperature of 37 degrees Celsius for 24 hours. Then, the diameter of the growth halo was measured with a caliper [29].

#### 2-6-5-Measurement of thermal properties (DSC)

DSC machine (Netzsch 200 F3 model made in Germany) was used in Urmia University to measure the thermal properties of the films. The accuracy of the device is 0.1 °C and it measures the transferred heat with an accuracy of 0.1 w/mg. The device was calibrated by indium and silver. Empty aluminum container was used as reference and nitrogen as atmosphere. Samples weighing 0.04 grams were heated at a speed of 22 °C/min in the temperature range of 0 to 240 degrees Celsius. From the obtained thermal pattern, the melting temperature (T<sub>m</sub>), the glass transition temperature (T<sub>g</sub>) was determined [29].

#### 2-6-6- Fourier transform infrared spectroscopy (FTIR)<sup>3</sup>

The changes of functional groups were investigated with the help of infrared Fourier transform spectroscopic device. In the resulting spectrum of the substance, the functional groups in the chemical structure appeared as peaks in specific areas. The FTIR spectrum was investigated in the transmission mode using a spectrophotometer in the wave number range of 4000-500 cm<sup>-1</sup> [29].

#### 2-7-Analysis of statistical analysis

In this study, the statistical method of the response surface and the central composite

statistical design were used to investigate the effect of two variable factors, the percentages of Avondol nanoparticles and milk thistle essential oil, on the physicochemical properties of the prepared films. The statistical analysis of the data was done at the probability level of 95% using Design Expert-10 software.

**Table 1:** Table of prepared films

Film	A: NP (%)	B: Essential oil (%)
F1	2	1
F2	4	2
F3	0	0
F4	0	2
F5	2	1
F6	4	1
F7	2	0
F8	2	1
F9	2	1
F10	0	1
F11	2	1
F12	4	0
F13	2	2

### 3- Results and Discussion

#### 3-1- Dynamic Light Scattering (DLS)

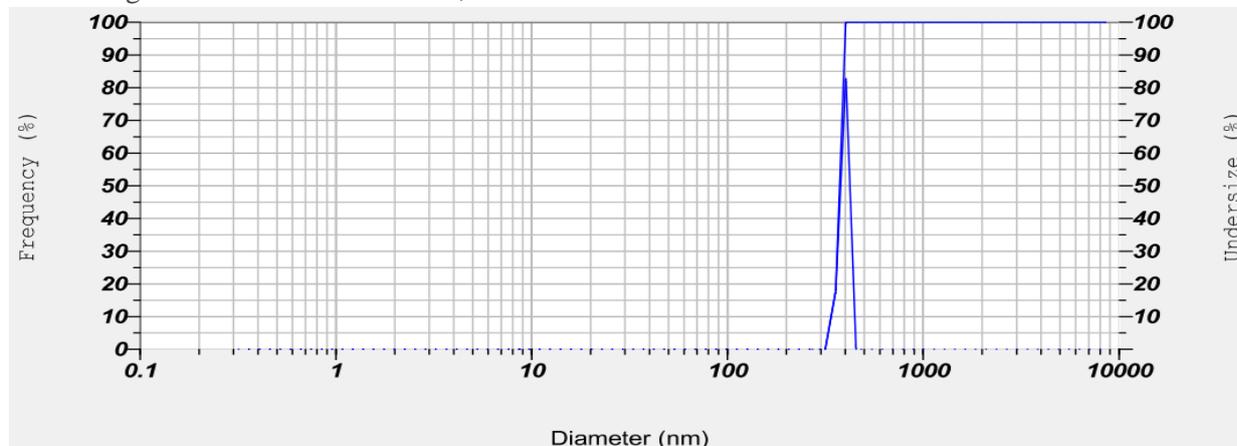
Particle size and zeta potential The particle size or size distribution was determined using dynamic light scattering (DLS) method, which is the most common technique for size distribution detection. Figure 3-1 shows the characteristics of nanopowders with a size of 371 nm. Zeta potential is a significant measure that indicates the stability of the micellar system. It has been reported that under a relatively high surface charge, the particles can repel each other with a strong electrostatic repulsion force, thereby increasing the stability of the system [31]. Zeta potential is a measure of the size of nanopowder charges. It has more value. In addition, researchers defined nanoparticle as a solid or dispersed particle with a scale of 1000 nm [32]. Microparticles is a

2- Tensile strength

3- Fourier transform infrared spectroscopy

term used to describe particles with a diameter of 1-1000 micrometers [33]. Many researchers used the term nanoparticle for their materials with sizes greater than 100 to 1000 nm, such as

150-250 nm [34], 234.7 nm-892.6 nm [35], 680 and 974 nm [36]. These results confirm that Avondol powder is a nanoparticle.



**Figure 3-1:** (DLS) alfalfa seed mucilage films with Smyrniun cordifolium nanoparticles and milk thistle essential oil

### 3-2- Color characteristics

The effect of the percentage of Avondol nanoparticles and milk thistle essential oil on L, a, b is shown in Figure 3-2. Mathematical equation shows the relationship between independent variables L, a, b and regression coefficients. Research in the field of food color and factors affecting it is still an important part of research in the field of food [37]. The color and transparency of packaging films play an important role in their appearance and acceptability. Usually, in the packaging of most food products, it is preferred that the polymer used is colorless, completely transparent and shows the appearance characteristics of the packaged product. The color parameter L provides a measure of lightness/darkness. The color parameter a provides a measure of redness/greenness, the color parameter b provides a measure of yellowness/blueness. L values range from 0 to 100 as an indication of dark to light. The higher the amount of the

compound, the whiter it is. According to the shapes, the amount of L did not change with the increase of Avondol nanodrate and milk thistle essential oil. Based on numerous studies, there are different results related to the effect of different nanoparticles on the color indices of nanocomposite films, which can be due to the different properties and characteristics of nanoparticles. Also, with the increase of Avondol nanoparticles and milk thistle essential oil, the amount of a and b decreased significantly ( $p < 0.05$ ), which is consistent with the results of Zulfi et al [38].

$$L^* = 83.999 - 97.113 *A - 78.379 *B + 127.597 *A*B$$

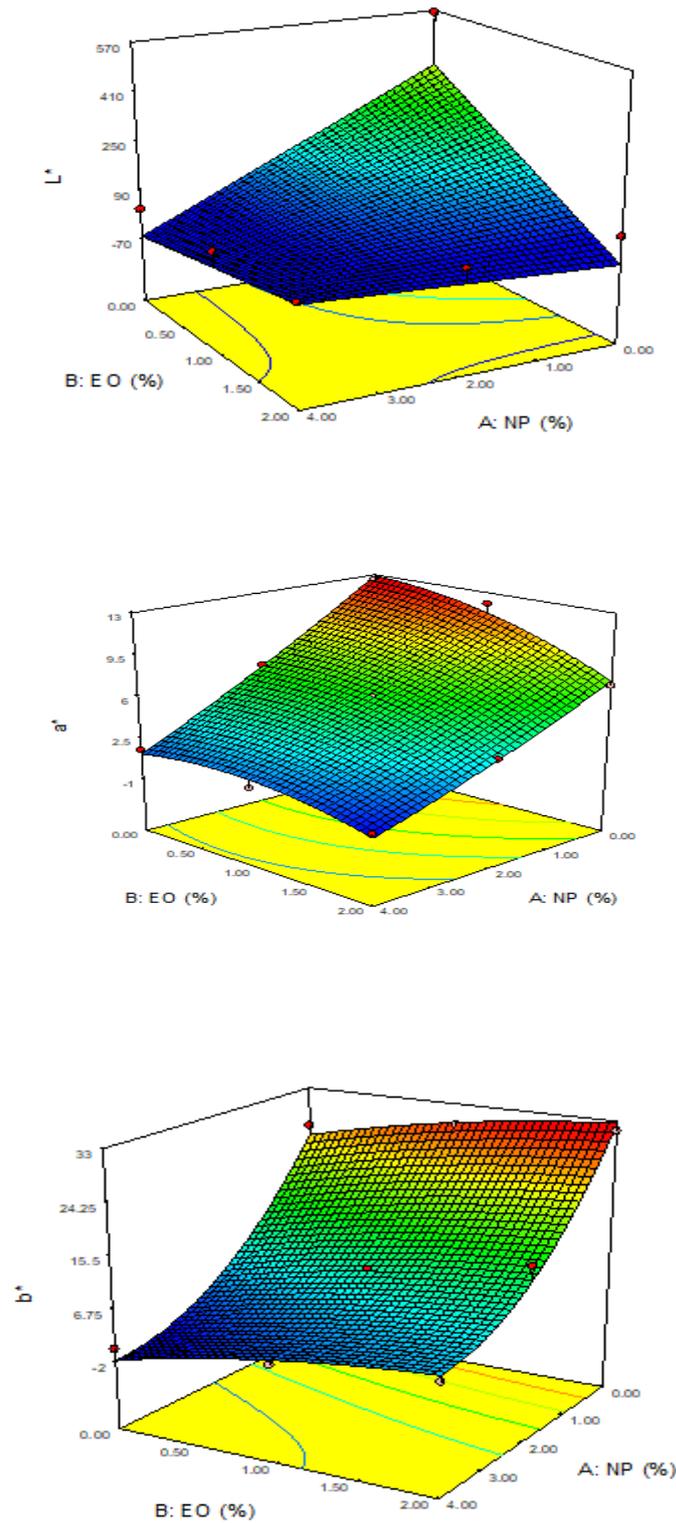
$$(R^2 = 0.634; \text{Adj}R^2 = 0.512)$$

$$a^* = 6.063 - 4.837 *A - 1.702 *B + 1.038 *A*B + 0.372 *A^2 - 1.106 *B^2$$

$$(R^2 = 0.990; \text{Adj}R^2 = 0.933)$$

$$b^* = 10.316 - 13.197 *A + 3.974 *B - 0.097 *A*B + 6.257 *A^2 - 1.178 *B^2$$

$$(R^2 = 0.978; \text{Adj}R^2 = 0.962)$$



**Figure 3-2:** L,a,b three-dimensional shape of alfalfa seed mucilage film with Smyrniun cordifolium nanoparticles and milk thistle essential oil.

**3-3- Mechanical properties of manufactured composites (tensile resistance, elongation percentage)**

One of the most important features of a synthetic film is its mechanical properties, this feature is affected by length The chain and

molecular weight of macromolecules, as well as the length and position of the side chains in it. These characteristics affect the biopolymer's ability to form intermolecular bonds. The more and stronger these bonds are, the greater the structural integrity and, as a result, the higher the mechanical resistance. Optimizing the mechanical properties of edible films is important from various aspects, such as the high strength of the film makes it not suffer from mechanical damage such as punctures due to the stress applied to the packaging material, and as a result, its inhibitory properties are better. To preserve gases and moisture. The high flexibility of the film makes it conform to the shape of the food item without breaking and can be easily used as a coating. Mechanical characteristics are among the important factors in choosing the type of food packaging. The packaging should be able to protect against physical stress and change in environmental conditions during storage until consumption. Tensile strength represents the maximum tensile stress a film can withstand, percent elongation represents a film's ability to stretch, it is the maximum change in length of a test specimen before breaking, and elastic modulus is a measure of film stiffness. Food packaging generally requires a lot of stress with deformation based on the desired application. TS is the maximum tensile stress endured by the sample during the tension test [39]. Figure

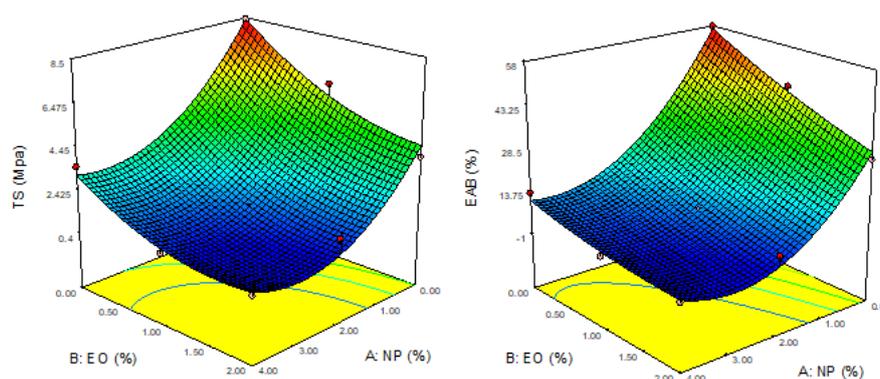
3 shows the results of the tensile strength and the percentage of elongation at the break point of the produced films. Tensile strength and elongation percentage for alfalfa seed mucilage film decreased by adding Avondol nanoparticles and milk thistle essential oil. Adding milk thistle essential oil to mucilage films reduces the tensile strength. This can be due to the disproportionate distribution of milk thistle essential oil in the polymer matrix of alfalfa seed mucilage, which is due to the lack of coordination between milk thistle essential oil and alfalfa seed mucilage biopolymer, which is due to the effect of fat on the branches of alfalfa seed and this phenomenon that the phase rich in Polysaccharide has a higher tensile strength compared to lipid phases, which is consistent with the results of Maizura et al [40].

$$\text{Tensile strength (Mpa)} = 1.636 - 2.199 * A - 1.545 * B + 0.439 * A * B + 1.804 * A^2 + 0.818 * B^2$$

$$(R^2 = 0.975; \text{Adj}R^2 = 0.958)$$

$$\text{Elongation at Break (\%)} = 9.265 + 27.34 - 18.586 * A - 8.387 * B + 4.527 * A * B + 14.559 * A^2 + 1.655 * B^2$$

$$(R^2 = 0.986; \text{Adj}R^2 = 0.977)$$



**Figure 3-3:** The three-dimensional figure of tensile strength, percent increase in film length of alfalfa seed mucilage with *Smyrnum cordifolium* nanoparticles and milk thistle essential oil.

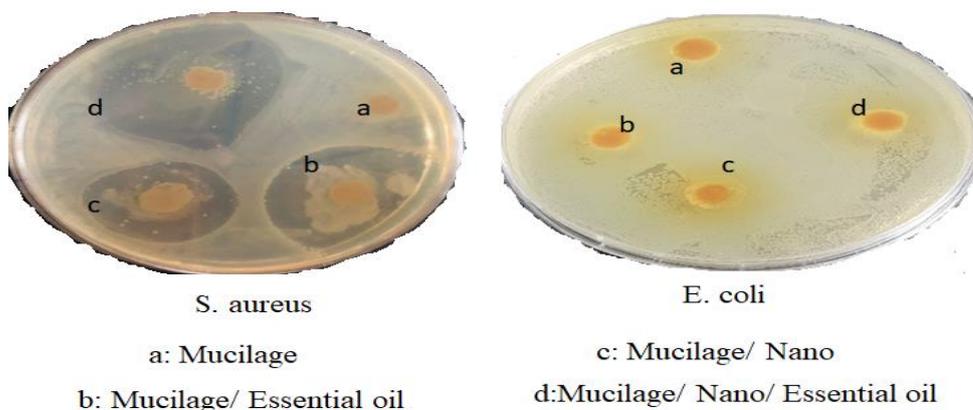
### 3-4- Investigation of antimicrobial properties

Antimicrobial properties of Avondol nanoparticle film and milk thistle essential oil are shown in table and figure 3-4. Edible films

can contain antioxidants and antimicrobial substances. For several years, food coatings have been considered to delay or prevent the growth of microorganisms. Microbial growth is largely a surface phenomenon, and therefore, by limiting the growth of bacteria on the surface of foods, the useful life will be improved. As it is known, the said films prevented the growth

of *Escherichia coli* and *Staphylococcus aureus*. With the increase in the amount of Avondol nanodrate and milk thistle essence, the diameter of the lack of growth has increased. Therefore, it can be said that films supported with these compounds can act as an active packaging against microorganisms. In general, the inhibitory property of films containing Avondol nanoparticles and milk thistle essential oil against Gram-positive microorganisms (*S. aureus*) is higher than Gram-negative types (*E. coli*). The cause of this phenomenon is the difference in the cell wall structure of these microorganisms. The main composition of the cell wall of gram-positive bacteria is peptidoglycan with a small amount of protein; However, the cell wall of Gram-negative bacteria is more complex despite being less thick and contains different polysaccharides, proteins and lipids in addition to peptidoglycan. Also, the cell wall of Gram-negative bacteria has an outer membrane that covers the outer surface of the wall. The combination of these factors increases the resistance of Gram-negative bacteria compared to Gram-positive bacteria [41]. Avondol

nanoparticles show different antibacterial properties based on the ratio of surface area to volume. It is possible that the ions released from nanomaterials react with thiol groups (SH-) of surface proteins of bacterial cells. A number of these bacterial cell membrane proteins are responsible for transferring minerals from the surface of the wall; that nanomaterials by acting on these proteins cause inactivation and impermeability of the membrane [42]. Inactivation of membrane permeability eventually causes cell death. Also, nanomaterials delay bacterial cell adhesion and biofilm formation, which prevents a group of bacteria from stabilizing and multiplying [43]. It has been found that the strong binding of nanoparticles to the outer membrane of bacteria can prevent the dehydrogenase enzyme from functioning. Preventing the activity of bacterial periplasmic enzymes and thus preventing the activity and function of RNA, DNA and protein synthesis has also been seen. In total, inhibition of these activities will lead to cell lysis [44].

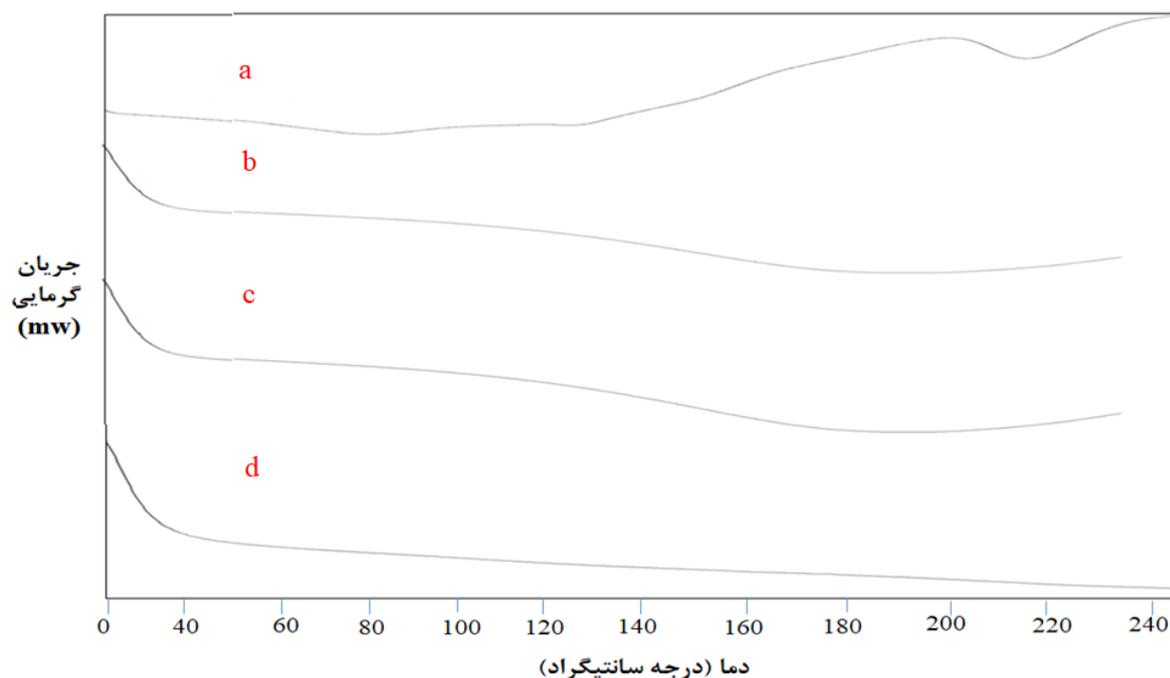


Microorganism		Films
E. coli	S. aureus	
-	3	<b>A</b>
-	22.12	<b>B</b>
-	17.03	<b>C</b>
-	31.21	<b>D</b>

**Figure 3-4:** The diameter of the growth halo (mm)**3-5- thermal properties (DSC)**

Thermal analysis of nanocomposite films based on alfalfa seed mucilage with avondol nanoparticles and milk thistle essential oil is shown in Figure 3-5. Investigating the thermal properties of films such as the glass transition temperature ( $T_g$ ) and melting temperature ( $T_m$ ) of the film is important, because the films are soft and rubbery at temperatures higher than the glass transition temperature and at temperatures higher than  $T_g$ . The reason for the increase in the space between the polymers, the mobility of the polymers increases and as a result the permeability of the film increases, so the higher the  $T_g$  of a film, the packaging film can act as a protector in a wider temperature range [45]. At the melting temperature, the polymer film becomes completely liquid. In the present study, the thermal properties of the produced films have been evaluated in terms of parameters such as glass transition temperature ( $T_g$ ) and melting temperature ( $T_m$ ). The results show that  $T_g$  increases with the addition of

Avondol nanoparticles and milk thistle essential oil, which confirms the strengthening role of Avondol nanoparticles and milk thistle essential oil in making nanocomposite films in a wide temperature range. The melting point temperature ( $T_m$ ) of the characteristics of the areas Crystalline and glass transition temperature ( $T_g$ ) depend on the characteristics of amorphous regions and these two temperatures do not necessarily increase or decrease together. According to the results, it was observed that by adding Avondol nanoparticles and milk thistle essential oil,  $T_g$  and  $T_m$  increased in nanocomposite films. This can be attributed to the establishment of strong interactions between the hydroxyl groups of the matrix and the nanofiller, as a result of which, the mobility and flexibility of alfalfa seed mucilage fibers, avondol nanoparticles, and milk thistle essential oil are reduced in the crystalline and amorphous regions, respectively.  $T_g$  and  $T_m$  increase [46]. Swaroop studies.



a: Mucilage    b: Mucilage/ Essential oil    c: Mucilage/ Nano    d: Mucilage/ Nano/ Essential oil

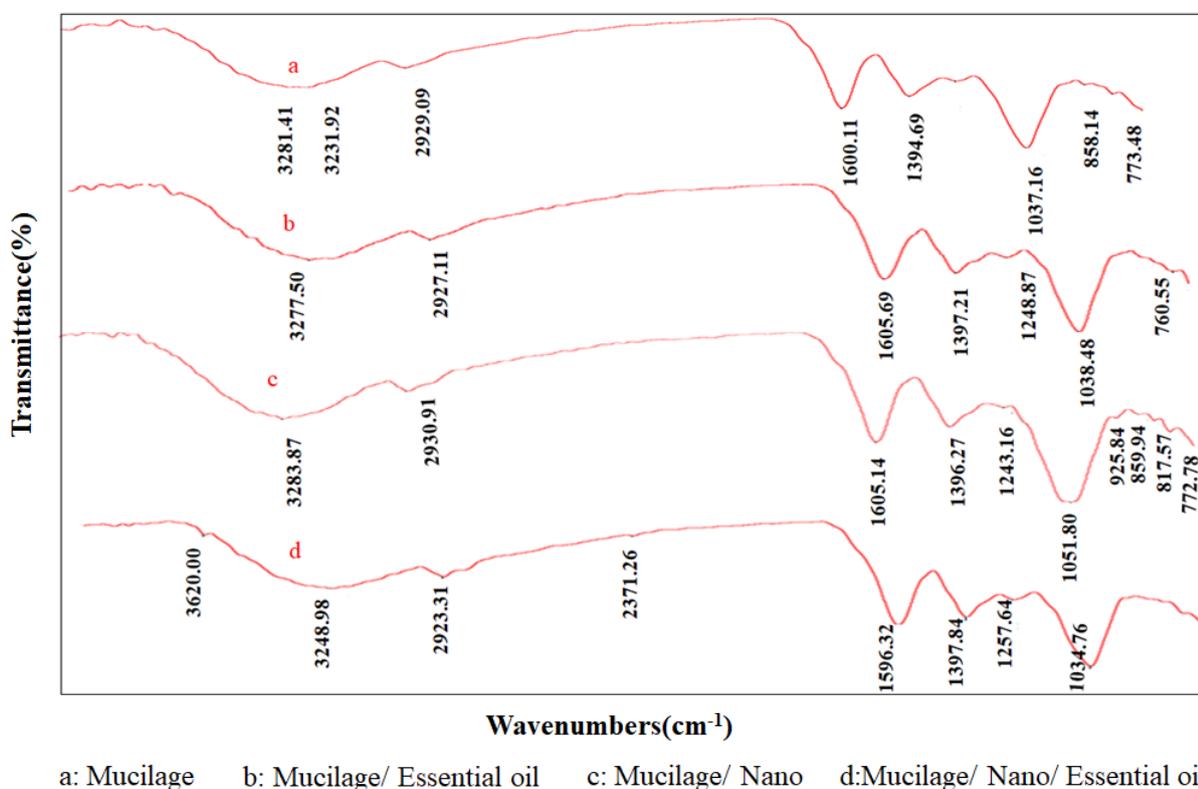
**Figure 3-5:** Measuring the thermal properties of alfalfa seed mucilage films with *Smyrniun cordifolium* nanoparticles and milk thistle essential oil.

**3-6- February infrared (FTIR)**

FTIR spectroscopy is often used as a suitable tool to determine specific functional groups or

chemical bonds present in a substance [48]. The results of infrared spectroscopy of alfalfa seed mucilage film and its nanocomposites are shown in Figure 3-6. The peaks between the wave number  $1, 3200$ , and  $3600$  are related to the OH stretching bands, which overlap with the NH stretching bands. The band appearing at  $1636 \text{ cm}^{-1}$  related to the vibration of carbonyl CO groups and the  $1500 \text{ cm}^{-1}$  band can be attributed to amide II groups, the  $1410 \text{ cm}^{-1}$  band related to the bending vibration of the OH group, the  $1142 \text{ cm}^{-1}$  peak related to the asymmetric stretching of the C O C bridge and the band Absorption at  $1090 \text{ cm}^{-1}$  can be attributed to skeletal stretching vibrations of  $1018 \text{ cm}^{-1}$  and O [49]. As it is known, the spectra of alfalfa seed mucilage are not much

different from its composites, because the functional groups in Avondol nanowires are similar to alfalfa seed mucilage, and therefore their peaks overlap with the alfalfa seed mucilage peaks and with Paying attention to the fact that the percentage of Avondol nanoparticles used in the structure of alfalfa seed mucilage film was not high, there was no noticeable change in the intensity of the peaks that appeared. The point that should be noted is that by adding milk thistle essential oil to the structure of the alfalfa seed mucilage film, the wave number of the appeared peaks has been shifted, and this shifting of the peaks indicates the creation of electrostatic interactions between the polymer chains of alfalfa seed mucilage and the essential oil. It is milk thistle.



**Figure 3-6:** FTIR results of alfalfa seed mucilage film with Smyrniun cordifolium nanoparticles and milk thistle essential oil.

#### 4 – Conclusion

The biocomposite of alfalfa seed mucilage, avendol nanoparticles and milk thistle essential oil as a biodegradable film with physical properties has a high potential as a coating. Milk thistle essential oil is also rich in antioxidant compounds. The composition of milk thistle essential oil has high antioxidant properties as well as high antimicrobial activity. Avondol nanoparticles and milk thistle

essential oil in combination with alfalfa seed mucilage can be used as a suitable coating for coating food. The effect of alfalfa seed mucilage biocomposite oral coating with Avondol nanoparticles and milk thistle essential oil was studied in terms of physicochemical properties. Despite all the advantages that alfalfa seed mucilage biopolymer has in the production of biodegradable films, but its poor mechanical

properties and sensitivity to water are the main obstacles to the widespread use of this biopolymer in the packaging industry. For this reason, alfalfa seed mucilage film with Avondol nanoparticles and milk thistle essential oil was used. The dynamic light scattering (DLS) results confirmed the Avondol nanoparticles. The results showed that with the increase of Avondol nanoparticles and milk thistle essential oil, tensile strength, elongation percentage and ab decreased. The addition of Avondol nanoparticles caused the antimicrobial activity of the film against *Staphylococcus aureus*. Avondol nanoparticles and milk thistle essential oil increase the thermal stability of alfalfa seed mucilage film. Fourier transform infrared (FTIR) results confirmed the physical presence of Avondol nanoparticles in the polymer matrix

## 5-References

- [1] P. Abdolsattari, S.H. Peighambaroust, S. Pirsaa, S.J. Peighambaroust and S.H. (2020).Fasihnia, nvestigating microbial properties of traditional Iranian white cheese packed in active LDPE films incorporating metallic and organoclay nanoparticles, *Chem Rev Lett* 3, 168–174.
- [2] S. Pirsaa, F. Mohtarami and S. (2020). Kalantari, Preparation of biodegradable composite starch/tragacanth gum/Nanoclay film and study of its physicochemical and mechanical properties, *Chem Rev Lett* 3, 98–103.
- [3] KarimiSani, S. Pirsaa and S.,Tagi,(2019). Preparation of chitosan/zinc oxide/*Melissa officinalis* essential oil nano-composite ~ film and evaluation of physical, mechanical and antimicrobial properties by response surface method, *Polym Test* 79, 106004.
- [4] L. Wu, L.L. Wang and H. Li, (2019). Two polyoxometalate-based coordination polymers: Synthesis, characterization and in vitro anti-lung cancer activity, *Main Group Chemistry* 18(4) , 337–344.
- [5] M. Pirouzifard, R.A. Yorghanlu and S. Pirsaa, (2020).Production of active film based on potato starch containing Zedo gum and essential oil of *Salvia officinalis* and study of physical, mechanical, and antioxidant properties, *J Thermoplast Compos* 33, 915–937.
- [6] S. Pirsaa, I. KarimiSani, M.K. Pirouzifard and A.(2020). Erfani, Smart film based on chitosan/*Melissa officinalis* essences/pomegranate peel extract to detect cream cheeses spoilage, *Food Add Contam A* 37 , 634–648.
- [7] S. Chavoshizadeh, S. Pirsaa and F. Mohtarami,(2020). Conducting/smart color film based on wheat gluten/chlorophyll/polypyrrole nanocomposite, *Food Packaging Shelf* 24 , 100501.
- [8] S. Asadi and S. Pirsaa,(2020). Production of Biodegradable Film Based on Polylactic Acid, Modified with Lycopene Pigment and TiO<sub>2</sub> and Studying Its Physicochemical Properties, *J Polym Environ* 28 , 433–444.
- [9] E. Farshchi, S. Pirsaa, L. Roufegarinejad, M. Alizadeh and M.(2019). Rezazad, Photocatalytic/biodegradable film based on carboxymethyl cellulose, modified by gelatin and TiO<sub>2</sub>-Ag nanoparticles, *Carbohydr Polym* 216 , 189–196.
- [10] Sothornvit, R. & Krochta, J. M. (2001). Plasticizer effect on mechanical properties of  $\beta$ -lactoglobulin films. *Journal of Food Engineering*, 50(3), 149-155.
- [11] Moghbel, A. & Tayebi, M. (2015). Quince Seeds Biopolymer: Extraction, Drying Methods and Evaluation.

- Jundishapur Journal of Natural Pharmaceutical Products, 10(3), e25392.
- [12] Tosif, M.M.; Najda, A.; Bains, A.; Kaushik, R.; Dhull, S.B.; Chawla, P.; Walasek-Janusz, M. A. (2021). Comprehensive Review on PlantDerived Mucilage: Characterization, Functional Properties, Applications, and Its Utilization for Nanocarrier Fabrication. *Polymers* 2021, 13, 1066. [CrossRef].
- [13] Bacenetti, J., Lovarelli, D., Tedesco, D., Pretolani, R., & Ferrante, V. (2018). Environmental impact assessment of alfalfa (*Medicago sativa* L.) hay production. *Science of the Total Environment*, 635, 551–558. <https://doi.org/10.1016/j.scitotenv.2018.04.161>.
- [14] Hojilla-Evangelista, M. P., Selling, G. W., Hatfield, R., & Digman, M. (2017). Extraction, composition, and functional properties of dried alfalfa (*Medicago sativa* L.) leaf protein. *Journal of the Science of Food and Agriculture*, 97(3), 882–888.
- [15] Cornara, L., Xiao, J., & Burlando, B. (2016). Therapeutic potential of temperate forage legumes: A review. *Critical Reviews in Food Science and Nutrition*, 56(sup1), S149–S161.
- [16] Anthony, K.; Saleh, M.A. (2012). Chemical profiling and antioxidant activity of commercial milk thistle food supplements. *J. Chem. Pharm. Res.*, 4, 4440–4450.
- [17] Flora, K.; Hahn, M.; Rosen, H.; Benner, K. (1998). Milk thistle (*Silybum marianum*) for the therapy of liver disease. *Am. J. Gastroenterol.* 93, 139–143. [CrossRef] [PubMed].
- [18] Deep, G.; Agarwal, R. (2007). Chemopreventive efficacy of silymarin in skin and prostate cancer. *Integr. Cancer Ther.* 6, 130–145. [CrossRef].
- [19] Pari, N., Parichehreh, Y., Alireza, R., & Naser, A. (2019). The role of *Smyrnum cordifolium* Boiss extract and curzerene on withdrawal syndrome in mice. *Cellular and Molecular Biology*, 65(7), 77-83.
- [20] Abbasi, N., Mohammadpour, S., Karimi, E., Aidi, A., Karimi, P., Azizi, M., & Asadollahi, K. (2017). Protective effects of *smyrnum cordifolium* boiss essential oil on pentylenetetrazol-induced seizures in mice: Involvement of benzodiazepine and opioid antagonists. *Journal of Biological Regulators and Homeostatic Agents*, 31(3), 683-689.
- [21] Tsai, W. T. (2013). Microstructural characterization of calcite-based powder materials prepared by planetary ball milling. *Materials*, 6(8), 3361-3372.
- [22] Baláž, M. (2018). Ball milling of eggshell waste as a green and sustainable approach: a review. *Advances in colloid and interface science*, 256, 256-275.
- [23] Broseghini, M., Gelisio, L., D’Incau, M., Ricardo, C. A., Pugno, N. M., & Scardi, P. (2016). Modeling of the planetary ball-milling process: The case study of ceramic powders. *Journal of the European Ceramic Society*, 36(9), 2205-2212.
- [24] Sipponen, M. H., Laakso, S., & Baumberger, S. (2014). Impact of ball milling on maize (*Zea mays* L.) stem structural components and on enzymatic hydrolysis of carbohydrates. *Industrial Crops and Products*, 61, 130-136.
- [25] Chaireh, S., Szécsényi, K. M., Boonsuk, P., & Kaewtatip, K. (2019). Preparation of rubber seed shell powder by planetary ball milling and its influence on the properties of starch

- foam. *Industrial Crops and Products*, 135, 130-137.
- [26] Wu, G.C.; Zhang, M.; Wang, Y.Q.; Mothibe, K.J.; Chen, W.X. (2012). Production of silver carp bone powder using superfine grinding technology: Suitable production parameters and its properties. *J. Food Eng.* 109, 730–735.
- [27] Jiang, C. Li, X. Jiao, Y. Jiang, D. Zhang, L. Fan, B. and Zhang, Q. (2014). Optimization for ultrasound-assisted extraction of polysaccharides with antioxidant activity in vitro from the aerial root of *Ficus microcarpa*. *Carbohydrate Polymers*, 110, 10-17.
- [28] Khakpour, F.; Pirsa, S.; Amiri, S. (2023). Modified Starch/CrO/Lycopene/Gum Arabic Nanocomposite Film: Preparation, Investigation of Physicochemical Properties and Ability to Use as Nitrite Kit. *Journal of Polymers and the Environment*.
- [29] Naiu, A.S.; Berhimpon, S.; Montolalu, R.I.; Kawung, N.J.; Suptijah, P.(2020). The effect of HCL-thermal pressure hydrolysis and high-speed destruction of chitin on particle size distribution and functional group of nano-chitin compound. *Curr. Res. Nutr. Food Sci.* 8, 197–205.
- [30] Liang, N.; Sun, S.; Li, X.; Piao, H.; Piao, H.; Cui, F.; Fang, L.(2012). Alpha-Tocopherol succinate-modified chitosan as a micellar delivery system for paclitaxel: Preparation, characterization and in vitro/in vivo evaluations. *Int. J. Pharm.* 423, 480–488.
- [31] Rizvi, S.A.A.; Saleh, A.M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharm. J.* 26, 64–70.
- [32] Stack, M.; Parikh, D.; Wang, H.; Wang, L.; Xu, M.; Zou, J.; Cheng, J.; Wang, H.(2018). Electro spun nanofibers for drug delivery. In *Electrospinning: Nanofabrication and Applications*; Elsevier Inc.: Amsterdam, The Netherlands, pp. 735–764.
- [33] Wang, J.; Tan, J.; Luo, J.; Huang, P.; Zhou, W.; Chen, L.; Long, L.; Zhang, L.; Zhu, B.; Yang, L.; et al. (2017). Enhancement of scutellarin oral delivery efficacy by vitamin B12-modified amphiphilic chitosan derivatives to treat type II diabetes induced-retinopathy. *J. Nanobiotechnol.* 15, 1–17.
- [34] Imansari, F.; Sahlan, M.; (2017). Arbianti, R. Release profile and inhibition test of the nanoparticles a. paniculata extract as inhibitor of  $\alpha$ -Glucosidase in the process of carbohydrates breakdown into glucose Diabetes mellitus. *IOP Conf. Ser. Mater. Sci. Eng. C.* 214, 012026.
- [35] El-Far, Y.M.; Zakaria, M.M.; Gabr, M.M.; El Gayar, A.M.; Eissa, L.A.; El-Sherbiny, I.M. (2017). Nanoformulated natural therapeutics for management of streptozotocin-induced diabetes: Potential use of curcumin nanoformulation. *Nanomedicine*, 12, 1689–1711.
- [36] MacDougall, D. B. (Ed.). (2002). *Colour in food: improving quality*. Woodhead Publ.
- [37] Zolfi, M., Khodaiyan, F., Mousavi, M., & Hashemi, M. (2014). The improvement of characteristics of biodegradable films made from kefiran–whey protein by nanoparticle incorporation. *Carbohydrate polymers*, 109, 118-125.
- [38] Srinivasan, M; Devipriya, N; Kalpana, K.B; and Menon, V.P.(2009). Lycopene: An antioxidant and radioprotector against radiation-induced cellular damages in cultured human lymphocytes. *Toxicol*, 262: 43-49.
- [39] Maizura F, Fazilah S, Norziah S and Karim B, (2007). Antibacterial activity and mechanical properties of partially hydrolyzed sago starch-alginate edible film containing lemongrass oil. *Journal of Food Science* 72: 324-330.
- [40] (41) Pranoto, Y, Rakshit, SK, & Salokhe, VM. (2005). Enhancing

- antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT-Food Science and Technology*, 38(8), 859-865.
- [41] .
- [42] Lin D H, Xing B S. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root elongation. *Environ. J Pollut.* 150:243-250.
- [43] Martel S.(2005). Method and system for controlling micro-objects or micro-particles. United States patent US 20100215785. *Appl.* 11/145,007.
- [44] King M D, Humphrey B J, Wang Y F, Kourbatova E V, Blumberg H M. Emergence of community-acquired methicillin-resistant staphylococcus aureus USA 300 clone as The predominant cause of skin and soft-tissue infections. *Ann Intern Med.* 2006;144:309-317.
- [45] Cao, X., Chen, Y., Chang, P.R., Stumborg, M. & Huneault, M.A. (2008). Green composites reinforced with hemp nanocrystals in plasticized starch. *Journal of Applied Polymer Science*, 109(6), 3804- 3810.
- [46] Zhang Y., Yu C., Hu P., Tong W., Lv F., Chu P.K., and Wang F., Mechanical and Thermal Properties of Palygorskite Poly(butylene succinate) Nanocomposite, *Appl. Clay Sci.*, **119**, 96-102, 2016.
- [47] Swaroop, C. & Shukla, M. (2019). Development of blown polylactic acid-Mg nanocomposite films for food packaging Composites Part A: Applied Science and Manufacturing.
- [48] Phisalaphong, M., & Jatupaiboon, N., (2008). Biosynthesis and characterization of bacteria cellulose–chitosan film. *Carbohydrate Polymers*, 74(3), 482-488.
- [49] Das, S., Das, M. P., & Das, J., (2013). Fabrication of porous chitosan/silver nanocomposite film and its bactericidal efficacy against multi-drug resistant (MDR) clinical isolates. *Journal of Pharmacy Research*, 6(1), 11-15.



مقاله علمی-پژوهشی

استخراج نانوذرات گیاه آوندول (*Smyrniun cordifolium Boiss*) و استفاده از آن در تهیه بیوکامپوزیت موسیلاژ بذر یونجه و روغن بذر گیاه خار مریم و بررسی خواص فیزیکوشیمیایی

فاطمه خاکپور<sup>۱\*</sup>، سجاد پیرسا<sup>۲</sup>

۱- دانشجوی کارشناسی ارشد گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

۲- استاد گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

اطلاعات مقاله	چکیده
تاریخ های مقاله :	زمینه مطالعاتی: افزودن نانوذرات آوندول و اسانس خار مریم می تواند سبب بهبود خواص فیزیکوشیمیایی فیلم های بر پایه موسیلاژ بذر یونجه شود. هدف: هدف در این مطالعه تهیه فیلم های خوراکی از موسیلاژ بذر یونجه با نانوذرات آوندول (۰، ۲، ۴٪) و اسانس خار مریم (۰، ۱، ۲٪) بود. روش کار: فیلم های خوراکی بر پایه موسیلاژ بذر یونجه تهیه شده و نانوذرات آوندول (۰، ۲، ۴٪) و اسانس خار مریم (۰، ۱، ۲٪) به آن افزوده شد. خواص فیزیکوشیمیایی فیلم ها تهیه شده بررسی شد. نتایج: مطابق نتایج به دست آمده با افزایش مقدار نانوذرات آوندول و اسانس خار مریم در فیلم باعث کاهش خواص مکانیکی فیلم ها شد. نتایج فعالیت ضد میکروبی نشان داد که افزودن نانوذرات آوندول باعث فعالیت فیلم علیه <i>استافیلوکوکوس اورئوس</i> شد. نتایج پراکنندگی نور دینامیکی (DLS) نانوذرات آوندول را تایید کردند. نتایج تبدیل فوریه فروسرخ (FTIR) حضور فیزیکی نانوذرات آوندول را در ماتریس پلیمری تایید کرد. افزایش نانوذرات آوندول و اسانس خار مریم در فیلم ها توانستند تجزیه حرارتی موسیلاژ بذر یونجه را به تاخیر بیاندازند و باعث افزایش پایداری حرارتی فیلم موسیلاژ گردند. نتیجه گیری نهایی: افزودن نانوذرات آوندول و اسانس خار مریم به فیلم های خوراکی بر پایه موسیلاژ بذر یونجه سبب بهبود ضد میکروبی، فوریه مادون قرمز (FTIR) فیلم ها، همچنین موجب تضعیف خواص مکانیکی گردید.
تاریخ دریافت: ۱۴۰۲/۷/۱۲	
تاریخ پذیرش: ۱۴۰۲/۹/۲۱	
کلمات کلیدی:	
فیلم خوراکی، موسیلاژ، نانوذرات آوندول، اسانس خار مریم.	
DOI: 10.22034/FSCT.21.147.114.	
مسئول مکاتبات: * sevdakhakpour1@gmail.com	