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FUNCTIONAL PROPERTIES, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ZATARIA MULTIFLORA ENCAPSULATED IN GELTIN NANOFILMS

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ABSTRACT

Development of biodegradable and biocompatible films based on the proteinpolymer with strong antibacterial activities is gradually obtained extensive concern in the world. The aim of this study was to evaluate the antioxidant and antibacterial properties of gelatin nano films merged with different concentrations of *Zataria multiflora* essential oil. Gelatin films were prepared from gelatin solutions (10% w/v) containing *Zataria multiflora* essential oil [ZMO] (2, 4, 6 and 8% w/w), glycerol (25% w/w) as plasticizer, and glutaraldehyde (2% w/w) as cross-linker. The mechanical, water solubility, water swelling, water vapor permeability, antioxidant and antibacterial properties of the films were measured according to the American Society for Testing and Materials. Gelatin films exhibited good tensile strength and elongation at break, water solubility, swelling, and water vapor permeability. Incorporation of ZMO into the gelatin films caused a significant decrease in tensile strength and swelling, and a significant increase in elongation at break, water solubility, water vapor permeability and whiteness of the films. Gelatin films exhibited low antioxidant activity while gelatin films incorporated with ZMO exhibited excellent antioxidant properties. The films incorporated with ZMO also exhibited excellent antibacterial properties against both Gram-positive and Gram-negative bacteria. Our results suggest that the gelatin/ZMO films could be used as a very attractive alternative to traditional materials for different biomedical applications.

Keywords: Gelatin film, *Zataria multiflora*, essential oil, antioxidants, antibacterial

INTRODUCTION

Gelatin is a soluble protein obtained by partial hydrolysis of collagen, the main insoluble fibrous protein constituent on bones, cartilages and skin **(Ktari *et al.*, 2014)**. Unique collagen and gelatin structures influence their physical properties, such as solubility, swelling, water uptake, moisture absorption, water evaporation, transparency, color, odor, gel strength, and thermal stability. Physical properties of gelatin itself influence gelatin quality and potential applications in food and pharmaceutical industries **(Gomez-Guillen *et al.*, 2002; Gómez-Guillén *et al.*, 2011)**. The food and pharmaceutical applications of gelatin are mainly based on gel-forming, film-forming and viscoelastic properties. Recently, an increasing number of new applications have been found for gelatin in products such as emulsifiers, foaming agents, colloid stabilizer, hydrogels, fining agents, packaging materials, wound dressing and micro-encapsulating agents **(Tharanathan 2003; Rawdkuen *et al.*, 2010)**. The highly hydrophilic nature of gelatin is its disadvantage when considering the use of gelatin films as protective barriers because they tend to swell or dissolve when contacting with the surface of foodstuffs with high moisture content. Consequently, the current trends in designing gelatin-based biodegradable materials for food packaging and medical applications are focused on developing films with improved mechanical and water resistance properties by combining gelatin with other biopolymer, synthetic polymer, plasticizer as well as cross-linker agents **(Bigi *et al.*, 2001; Marsh and Bugusu, 2007; Zhao *et al.*, 2008; Cao *et al.*, 2009; Bajpai *et al.*, 2013)**.

Gelatin is reported as one of the first carriers of bioactive components. Enrichment of gelatin films with natural antioxidant and/or antimicrobial substances will extend the functional properties of these biodegradable films and provide active packaging biomaterials. There is growing interest in using plant extracts as natural sources of antioxidant/antibacterial compounds in formulating gelatin films **(Appendini and Hotchkiss, 2002; Gomez-Guillen *et al.*, 2009; Hanusova *et al.*, 2009; Lucera *et al.*, 2012)**. In this context, plant essential oils and their main components are gaining a wide interest in health industry for their potentials as antioxidant and antimicrobial agents, since they are generally recognized as safe (GRAS) **(Tajkarimi *et al.*, 2010; Solorzano-Santos and Miranda-Novales, 2012)**.

To the author's best knowledge, there is no report on the antioxidant and antimicrobial activities of gelatin films incorporated with *Zataria multiflora* (ZM). ZM is a thyme-like plant belonging to the *Lamiaceae* family that grows only in Iran, Pakistan and Afghanistan. This plant has played an important role in Iranian traditional medicine. It has several traditional uses as an antiseptic, carminative, stimulant, diaphoretic, diuretic, anesthetic, antispasmodic and analgesic. In the modern pharmacological and clinical investigations, ZM is a valuable medicinal plant that has anti-microbial, antioxidant, anti-inflammatory, spasmolytic and anti-nociceptive properties.

In this study the gelatin films with antioxidant and antimicrobial activities were utilized from gelatin solutions containing different *Zataria multiflora* essential oil (ZMO) concentrations. The mechanical, water solubility, water swelling, water vapor permeability, color properties of gelatin/ZMO films and antioxidant and antibacterial activities of the gelatin/ZMO were examined.

MATERIAL AND METHODS

Preparation of gelatin films

To cast the films, 10 mL of gelatin film forming solutions containing different ZM concentrations were transferred into the polyester Petri dish (Farazbin Kimia Co., Teheran, Iran, the radius of 74mm) and placed at room temperature until the films dried. The obtained dry films were peeled off and stored until analysis. Thicknesses of films were measured to the nearest 0.01 mm with a digital micrometer (The L.S. Starrett Co. LTD. Great Britain, Uk) and the average was taken 120 ± 5μm.

Mechanical properties of films

The mechanical properties of the gelatin films were measured according to the American Society for Testing and Materials 638-02a (ASTM D 638-02a)

(ASTM, 2002). Gelatin films containing different concentrations of ZMO were transferred to a closed container with relative humidity of 65% (saturated sodium nitrite vapor) and left for equilibrium for 48 hours before mechanical testing. Gelatin films were cut into the rectangles with length of 60 mm, width of 10 mm and thickness 0.12 mm. The tensile strength test was then performed by stretching the film at pretest, test and posttest speeds of 1, 1, and 10 mm/s, respectively. The net length between the jaws for all films was almost constant at about 20 mm. The texture analyzer ran at auto force mode with the trigger force of 5gr (0.049 Newton). From stress-strain curves, two parameters were measured: 1) tensile strength (TS) was measured as maximum stress and 2) elongation at break (EAB) where the film was torn **(Gomez-Guillen *et al.*, 2002; Ahmad *et al.*, 2012)**:

TS (N/m^2) = (Breaking force / Cross-sectional area of sample)

EAB (%) = [(Increase in length at breaking point / Initial length)] × 100.

The area of film used for each experiment was 6 × 1 cm^2. However, 2 cm of the films were within the jaws, therefore the initial length of the film was taken as 4 cm^2. All tests were the means of at least three measurements.

Water solubility of films

To determine solubility, one piece of films (60 mm × 10 mm × 0.11 mm) was placed in an oven at 104 °C for 24 hours and initial dry weight (W_i) was calculated. Then, the dried films were immersed into a 100 mL Erlenmeyer flask containing 50 mL of distilled water and placed inside the shaker for 24 hours at 25 °C (Incubator with inventilator, Pars Azma Co. Tehran, Iran). Thereafter, the films were taken out and transferred to the oven of 104 °C for 24 hours and the final dry weight (W_f) was calculated. The weight loss or solubility percentage (S%) was determined by the following formula: S (%) = [(W_i-W_f) / W_i] × 100 (Gomez-Guillen, *et al.*, 2002, Ahmad, *et al.*, 2012). All tests were the means of at least three measurements.

Swelling of films

The gelatin films were dried in an air-circulating oven at 104 °C for 24 hours until reaching the constant weight before swelling test (W_i). Squares were cut with the dimensions of 20 mm × 20 mm × 0.12 mm for the swelling experiment. Each sample was immersed into a 100 mL Erlenmeyer flask containing 50 mL of distilled water. The samples were kept at room temperature during the swelling experiment (24 hours). Each sample was taken out of the flask after 24 hours, wiped between filter papers to remove the excess surface water and weighed (W_f). The gained weight or swelling percentage (SW %) was calculated by the following equation (Gomez-Guillen, *et al.*, 2002, Ahmad, *et al.*, 2012): SW (%) = [(W_f- W_i)/ W_i] × 100. All tests were the means of at least three measurements.

Water vapor permeability of gelatin films

The water vapor permeability (WVP) of the films was determined according to the ASTM E96-95 method (ASTM, 1995). The films were conditioned for 24 hours at 25 °C and 75% relative humidity before WVP determination. Film samples were mounted on an aluminum cup (height and diameter were 2.1 and 5.6cm, respectively). The cup was filled with 20 g of silica gel and covered with a film specimen. The cup was placed at 25 °C and 75% relative humidity in desiccators. The weight of the cup was measured at 3 hours intervals during one day. Simple linear regression was used to estimate the slope of mass change vs. time plot. The WVP was calculated by the following formula **(Gomez-Guillen *et al.*; 2002, Ahmad *et al.*, 2012)**: WVP ($g.mm/m^2.kPa.h$) = [(WVTR × T)]/ ΔP. Where water vapor transmission rate (WVTR) is the slope per film area ($g/m^2.h$), T is the film thickness (mm), and ΔP is the partial water vapor pressure difference (kPa) between the two sides of the film (4.2449 kPa at 30 °C).

Antioxidant activity of films

Antioxidant activities of the films were determined by decolorization method with 2, 2′-azino-di (3-ethylbenzthiazoline-6-sulfonate) (ABTS, Sigma, Germany) **(Tongnuanchan *et al.*, 2012)**. The method was modified to detect the continuous antioxidant release from films. The release tests were performed in 24 well plates. Briefly, cuts (10 mm × 10 mm × 0.12 mm, 20 mg) from different parts of the films containing 1, 2, 5, and 10% of the ZMO were added to 2.0 mL of diluted ABTS radical solution (7 mM ABTS and 2.54 mM potassium persulfate, A734 = 1 ± 0.1). Films without ZMO were used as blank. The program was adjusted to record the absorbance values after shaking the 24 well plates for 30s by plate reader (BioTekElx 808, Winooski, VT, 05403, USA). The data were recorded up to the steady state was reached for each sample. A standard curve of ascorbic acid ranging from 0.44 to 15.76 mg/mL was prepared. Antioxidant activity was expressed as mg ascorbic acid equivalents per gram of films using the standard curve.

Antibacterial activity of films using disc diffusion

All microorganisms obtained from the Persian Type Culture Collection (PTCC), Tehran, Iran. The films were individually tested against two Gram-negative bacteria [*P. aeruginosa* PTCC 1074 (ATCC 9027) and *E. coli* PTCC 1330 (ATCC 8739)] and two Gram-positive bacteria [*S. aureus* PTCC 1112 (ATCC 6538) and *B. subtilis* PTCC 1023 (ATCC 6633)]. The antibacterial activity was measured according to the standard practice for determining resistance of synthetic materials to bacteria (ASTM G22-76) **(ASTM, 1996)**.

To investigate the antimicrobial activity of the films by disc diffusion, 30 mm diameter discs (thickness of 0.12 mm) were cut from different parts of the films and sterilized by autoclaving for 30 min at 120 °C **(Bauer *et al.*, 1966)**. Bacterial suspensions with a turbidity equivalent to a 0.5 McFarland standard were prepared (108 colony-forming units CFU/mL) and then diluted to 105 CFU/mL with Luria-Bertani Broth (LB). The adjusted bacterial suspensions (0.1 mL) were spread on to the nutrient agar plates (Farazbin Kimia Co., Teharan, Iran) containing LB. Subsequently, the discs were placed in direct contact with the agar medium. Plates were inverted and incubated at 37 °C for 24 hours (Incubator with inventilator, Pars Azma Co. Tehran, Iran). Films without ZMO under the same condition were used as the controls. The diameters of clear inhibition zones, including the diameter of the disc, were measured with ruler and the results were used to evaluate antibacterial potential of the films.

Antibacterial activity of gelatin films using colony counting

The bacterial colony counting assays were conducted according to the Clinical and Laboratory Standards Institute (CLSI) and ASTM G22-76 **(ASTM, 1996)**. Bacteria strains were suspended in LB media and the densities were adjusted to 0.5 McFarland standards at 640 nm (108CFU/ mL) and then diluted to 105 CFU/mL with LB. A sample film with 30 mm diameter was placed in a 10 mL liquid culture containing 10 µL of the cultures. Then, the sample was incubated at 37 ºC for 24 hours (Shaking Incubator, Shin Saeng, Fine Tech, Korea). From the incubated samples, a 100 µL solution was taken and diluted with the appropriate dilution factor and the final diluted microbe solution, and then it was plated and distributed onto the nutrient agar plates (Farazbin Kimia Co., Tehran, Iran). The plates cultured with the films without ZMO under the same condition were used as the controls. All plates were incubated at 37 ºC for 24 hours and the number of formed colonies was counted. The antibacterial efficacy of the films was calculated by the following equation (Maneerung, *et al.*, 2008): Colony reduction (%) = [(Number of colonies in the test samples - Number of colonies in the control)/ Number of colonies in the test samples] × 100.

Statistical analysis

Data were expressed as the means ± standard deviations of at least three independent experiments. The significant differences between the treatments were analyzed by one-way analysis of variance (ANOVA) and Duncan tests at $P < 0.05$ by SPSS version 18 (SPSS18, Abaus Concepts, Berkeley, CA) and Prism 5 (Graph Pad, San Diego, USA) softwares.

RESULTS AND DISCUSSION

Dietary proteins are a source of biologically active peptides, which are inactive in the parent protein sequence but can be liberated during gastrointestinal digestion, food processing or fermentation. Once they are released, bioactive peptides can affect numerous physiological functions of the organism. Gelatin has been focused on as a source of biologically active peptides with promising health benefits for nutritional or pharmaceutical applications. Most of the studies about gelatin-derived peptides in the area of food science and technology have dealt with their antioxidant, antibacterial and antihypertensive inhibitory activity. In this study we examined potential of antioxidant/antibacterial activities of gelatin films incorporate with ZMO as a wound dressing was investigated

Mechanical properties of films

The mechanical properties of gelatin films are shown in Figures 1 and 2. Tensile strength and elongation at break are parameters that relateed to mechanical properties of films and this property is related to their chemical structures. Tensile strength and elongation at break of gelatin films cross-linked with glutaraldehyde were 4.2 ± 0.4 MPa and 128 ± 7%, respectively. Incorporation of ZMO into gelatin films caused a significant decrease in tensile strength (from 3.7 ± 0.21 to 2.6 ± 0.13 MPa) and increase in elongation at break (from 139 ± 6% to 165 ± 7%) ($P < 0.05$). Gelatin films were mainly stabilized by the weak bond including hydrogen bond and hydrophobic interaction **(Bigi *et al.*, 2001)**. However, ZMO incorporation especially at higher concentrations caused a significant decrease in tensile of the films. Addition of ZMO possibly resulted in lowering the interaction between gelatin monomers, and hindering the polymer chain-to-chain interactions, which consequently caused a decrease in tensile with the simultaneous increase in elongation at break of the films **(Limpisophon *et al.*, 2010)**. Gelatin films incorporated with citrus oil showed lower tensile

strength but higher elongation at break than the control films free of incorporated essential oil, similar to the results of the current study **(Tongnuanchan *et al.*, 2012)**. These results suggested that gelatin films incorporated with ZMO could be promising candidates for physically powerful food packaging.

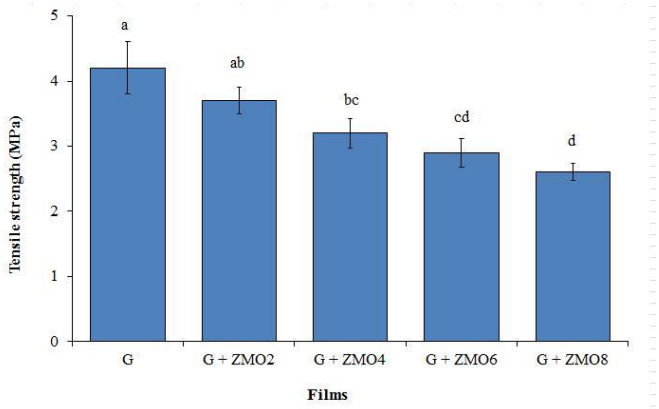

Figure 1 Tensile strength (MPa) of gelatin (G) films as function of *Zataria multiflora* essential oil (ZMO). ZMO2, ZMO4, ZMO6 and ZMO8 are 2%, 4%, 6% and 8% w/w ZMO based on the gelatin powder. Different letters show significant difference (P< 0.05).

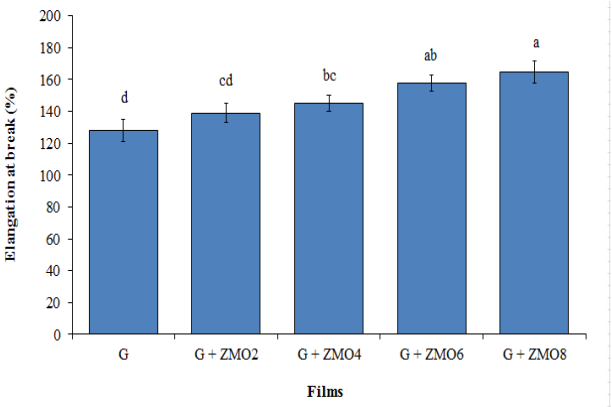

Figure 2 Elongation at break (%) of gelatin (G) films as function of *Zataria multiflora* essential oil (ZMO). ZMO2, ZMO4, ZMO6 and ZMO8 are 2%, 4%, 6% and 8% w/w ZMO based on the gelatin powder. Different letters show significant difference (P< 0.05).

Solubility determination of films

The solubility percentages (weight loss) of gelatin films are summarized in Figure 3. The solubility percentage of gelatin films was 29 ± 1.6%. Incorporation of ZMO into the films caused a significant increase in the solubility (From 30 ± 1.3% to 37 ± 1.6%) of gelatin films, dose-dependently (P < 0.05). Gelatin is water-soluble, can easily dissolve partially when coming into contact with an aqueous medium, and may lose fibrous structure to high ambient humidity especially for long periods of time. However, cross-linking can stabilize gelatin structure and decrease its solubility in aqueous mediums **(Bigi *et al.*, 2001)**. Generally, the effects of the additives on the solubility of films depend on the type of compounds and their hydrophilicity and hydrophobicity indexs **(Rhim *et al.*, 2000)**. ZMO is a hydrophobic material that favorably interacts with hydrophobic domain of gelatin and may hinder gelatin network formation and consequently cause an increase in the solubility of the films **(Hong *et al.*, 2009)**. Gelatin-chitosan films in the presence of essential oil, showed a significant increase in the film solubility, similar to the results of the current study **(Gomez-Estaca *et al.*, 2010)**. These results recommended that gelatin films incorporated with ZMO could be promising candidates for water resistant food packaging.

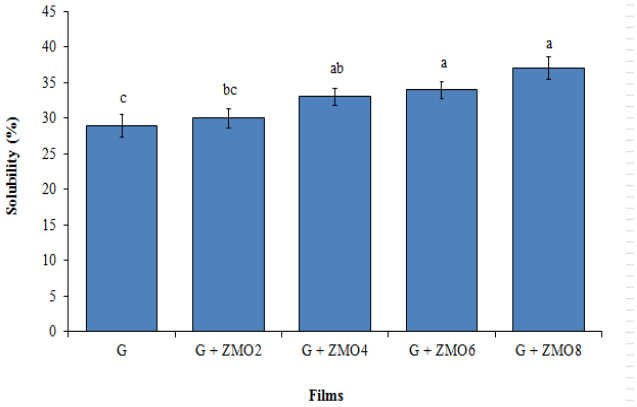

Figure 3 Solubility (%) of gelatin (G) films as function of *Zataria multiflora* essential oil (ZMO). ZMO2, ZMO4, ZMO6 and ZMO8 are 2%, 4%, 6% and 8% w/w ZMO based on the gelatin powder. Different letters show significant difference (P< 0.05).

Swelling capacity of films

The results of swelling capacity of gelatin films are summarized in Figure 4. The swelling percentage for gelatin films was 396 ± 8%. Incorporation of ZMO into the films caused a significant decrease in the swelling (from 381 ± 9% to 344 ± 5%) (P < 0.05). Gelatin is a hydrophilic material expected to absorb molecules of water. The porous gelatin films showed higher swelling capacity because of the porosity in their network structures that allows more water to enter inside the film **(Avena-Bustillos *et al.*, 2011)**. Incorporation of ZMO could reduce swelling capacity of the films which might be related to hydrophobic nature of ZMO. Hydrophobic domains of gelatin can essentially interact with ZMO through hydrophobic interaction and thereby enhance interfacial interaction between matrix (gelatin) and filler (ZMO) **(Zivanovic *et al.*, 2005, Hong *et al.*, 2009)**. This event saturated gelatin network with ZMO and water molecules could not diffuse to gelatin network, thereby swelling decreased. These results recommended that gelatin films incorporated with ZMO could be promising candidates for water resistant food packaging.

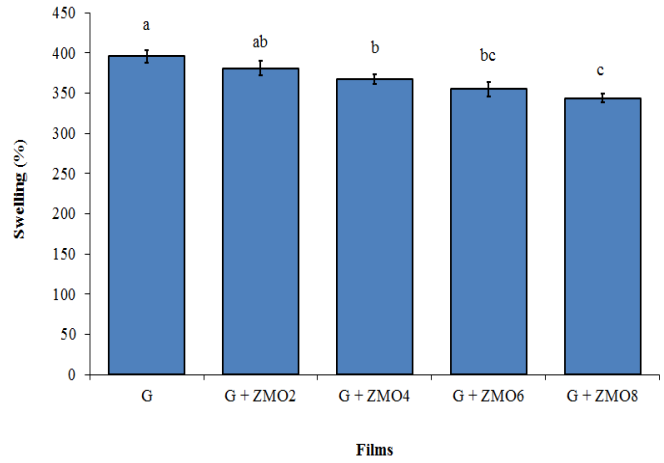

Figure 4 Swelling (%) of gelatin (G) films as function of *Zataria multiflora* essential oil (ZMO). ZMO2, ZMO4, ZMO6 and ZMO8 are 2%, 4%, 6% and 8% w/w ZMO based on the gelatin powder. Different letters show significant difference (P< 0.05).

Water vapor permeability of films

The results of water vapor permeability (WVP) of gelatin films are summarized in Figure 5. The WVP for gelatin films was 0.23 ± 0.018 g.mm/kPa.m^{2}h. Incorporation of ZMO into the films caused a significant increase in WVP (from 0.24±0.012 to 0.32±0.015 g.mm/kPa.m^2.h) of the films, dose-dependently (P < 0.05). Gelatin is a hydrophilic material that strongly interacts with water molecules and causes a reduction in the water vapor transmission through gelatin films **(Rojas-Graü *et al.*, 2007; Zhang *et al.*, 2007; Avena-Bustillos *et al.*, 2011)**. Incorporation of the additive to gelatin films causes a significant change in water vapor transmission through films, while the final WVP capacity is related to hydrophobicity/hyrophilicity index of all compounds in the films. Hydrophobic domains of gelatin can essentially interact with ZMO through hydrophobic interaction and thereby enhance interfacial interaction between matrix and ZMO. This phenomenon hinders interactions between gelatin chains and water molecules, thus water molecules freely pass through the films and

consequently cause the increase in WVP **(Zivanovic *et al.*, 2005; Hong *et al.*, 2009)**. The WVP of chitosan films incorporated with thyme oil slightly increased, similar to the results of the current study **(Altiok *et al.*, 2010)**. These results recommended that gelatin films incorporated with ZMO could be promising candidates for water barrier food packaging.

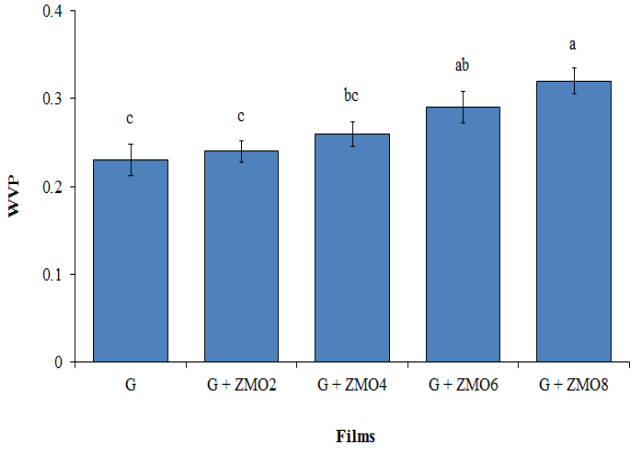

Figure 5 Water Vapor Permeability (WVP) (g.mm/m^2.kPa.h) of gelatin (G) films as function of *Zataria multiflora* essential oil (ZMO). ZMO2, ZMO4, ZMO6 and ZMO8 are 2%, 4%, 6% and 8% w/w ZMO based on the gelatin powder. Different letters show significant difference (P< 0.05).

Antioxidant activity of films

Antioxidant activity of the gelatin films incorporated with different ZMO concentrations was determined by ABTS decolorization method and expressed as mg ascorbic acid equivalent per gram of films (Figure 6). The gelatin films free of ZMO showed very low activity against the ABTS decolorization. Various studies have examined antioxidant properties of peptides derived from gelatin in different sources. These studies have shown that peptides derived from enzymatic hydrolysis of gelatin are lipid peroxidation inhibitors, free radical scavengers, and transitional metal ion chelators. The anti-oxidative properties of peptides are related to their amino acid composition, molecular weight, structure, and hydrophobicity **(Aleman *et al.*, 2011; Gómez-Guillén *et al.*, 2011)**. The gelatin films containing different ZMO concentrations decolorize ABTS dose-dependently. Fish skin gelatin films incorporated with citrus essential oils **(Tongnuanchan *et al.*, 2012)** and chitosan films incorporated with thyme oil (Altiok, *et al.*, 2010) exhibited strong antioxidant activity, similar to the results of the our study. These antioxidant activities may be attributed, at least in part, to the presence of phenols, flavonoids, sesquiterpenes and sulfur-containing compounds in the ZMO **(Nazari and Iranshahi 2011, Kavoosi and Rowshan 2013)**. These results recommended that gelatin films incorporated with ZMO could be promising candidates for safe radical scavenger food packaging. Such antioxidant activities could diminish oxidative damage in foodstuff.

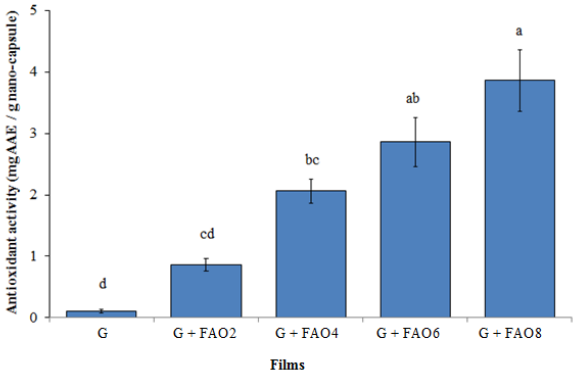

Figure 6 Antioxidant activity of gelatin (G) films incorporated With *Zataria multiflora* essential oil (ZMO). ZMO2, ZMO4, ZMO6 and ZMO8 are 2%, 4%, 6% and 8% w/w ZMO based on the gelatin powder. The antioxidant activity was expressed in milligram ascorbic acid equivalent (AAE) per gram incorporating different concentrations of ZMO. Mean values with different letters within a column are significantly different by Duncan's multiple range tests at (P < 0.05).

Antibacterial activity of gelatin films

Antibacterial assay of gelatin films incorporated with ZMO expressed by disc diffusion method and viable colony counting assay. The results of disc diffusion are summarized in Figure 7. The initial diameter of all films was fixed at 30 mm. The diameters of clear inhibition zones, including the diameter of the disk, were used to analyze the antibacterial activity. According to the results, all gelatin films free of ZMO showed no activity against the tested bacteria while the antibacterial activity of gelatin films containing different ZMO concentrations was the highest against *B. subtilis* and *S. aureus* followed by *E. coli* and then *P. aeroginosa*. According to the Swiss Norm (SN) 195920-ASTM E 2149-01, any agent showing zone inhibition of > 1 mm, is considered as a good antibacterial agent **(Pinto *et al.*, 2009)**. Thus gelatin films incorporated with ZMO are effective against both Gram-positive and gram-negative bacteria while they have more effect on Gram-positive bacteria. The results of colony reduction percentage are summarized in Figure 8. According to the obtained results, the antibacterial activity of gelatin films containing different ZMO concentrations was highest against *S. aureus* followed by *B. subtilis*, *E. colli*, and then by *P. aeroginos*a.

The antibacterial activities recognized in the essential oils from several medicinal plants indicated that the essential oils are attacked to the phospholipids in the cell membranes, which cause increased permeability and leakage of cytoplasm, or in their interaction with enzymes located on the cell wall. Thus, the resistance of Gram-negative bacteria to the essential oils likely lies in the protective role of their cell wall lipopolysaccharide or outer membranes proteins, which restrict diffusion of hydrophobic compounds through the lipopolysaccharide layer **(Oussalah *et al.*, 2007)**. Essential oils have the ability to disrupt lipid structure of the bacteria cell walls, leading to destruction of cell membrane, cytoplasmic leakage, cell lysis and ultimately cell death. The decrease in pH that occurs due to cell membrane disruption resulted in a loss of control of cellular processes such as ATP biosynthesis, DNA transcription and protein synthesis **(Xu *et al.*, 2008)**. Essential oils also penetrate into mitochondrial membrane, leading to the greater permeability of organelle and the potassium ion leakage process. The leakage of ions, especially potassium, out of a cell is a clear indication of membrane damage and cell death **(Paparella *et al.*, 2008)**. ZMO gradually released from films to the solution and penetrated into the cell membranes and disrupted the membrane structure and finally caused cell death.

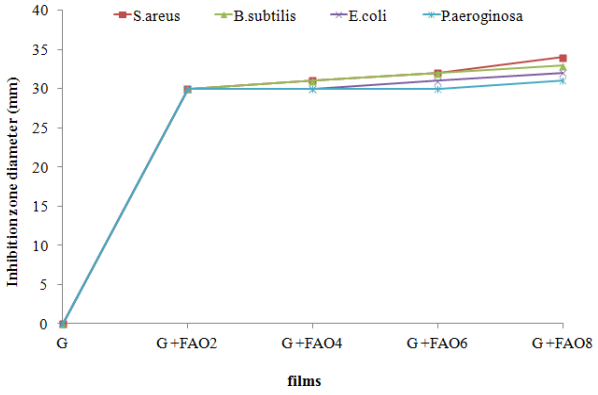

Figure7 Antibacterial activity of gelatin (G) films incorporated with *Zataria multiflora* oil (ZMO) by disc diffusion method. Antibacterial activity was expressed as diameter of bacterial growth inhibition zone in the presence of films with different ZMO concentrations. Mean values with different letters within a column were significantly different by Duncan's multiple range tests at (P < 0.05).

CONCLUSION

We explained the gelatin nano films merged with ZMO have excellent physical, well as good antioxidant and antibacterial activities, which could be good candidates for safe antimicrobial food packaging. So this fact could diminish growth of pathogenic bacteria in the foodstuff and could be used in food industry.

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