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Evaluation of antimicrobial activity of Poly Lactic Acid (PLA) films containing cellulose nanoparticle and *Bunium persicum* and *Mentha piperita* essential oils (EOs)

Talebi, F., Misaghi, A.*, Khanjari, A., Kamkar, A., Gandomi, H., Saeedi, M.

Department of Food Hygiene and Qualty Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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Correspondence
Misaghi, A.
Department of Food Hygiene and Qualty Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Tel: +98(21) 61117040
Fax: +98(21) 66438141
Email: amisaghi@ut.ac.ir

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Abstract:

BACKGROUND: Packaging is one of the important aspects of food quality and safety. Unfortunately, most food packaging materials are from oil resources that are limited resources and undegradable. **OBJECTIVES:** The aim of this study was reaching an environmentally friendly packaging along with enhancement of food safety. **METHODS:** After obtaining *Bunium persicum* (BP) and *Mentha piperita* (MP) essential oils (EOs) by steam distillation method, their chemical compositions were determined using GC-MS analysis. PLA films were prepared using solvent casting technique containing different concentrations of BP (0, 0.5 and1%v/v), MP (0, 0.5 and1%v/v) EOs and cellulose nanoparticle (CN) (0 and 1% w/v) and their antimicrobial effects against gram positive bacteria (*Staphylococcus aureus* ATCC 65138, *Bacillus cereus* ATCC 11778) and Gram negative bacteria (*Vibrio parahaemolyticus* ATCC 43996, *Escherichia coli* O157:H7 and *Salmonella typhymurium* ATCC 14028) were assessed by disk diffusion method. **RESULTS:** Major compounds of BP EO were Propanal 2-methyl-3-phenyl (34.08%), Cymene (18.23%) and Myrtenal (12.37%) and for MP EO were p-Menthan-3-ol (44.59%) and p-Menthan-3-onetrans (12.14%). The results of present study indicated that pure PLA film or PLA films containing CN showed no antimicrobial activity against any of the five tested bacteria but films containing EOs had significant antimicrobial activity and BP EO was more effective than MP EO and their combination ($p<0.05$). Besides, the inhibitory effect of films was concentration-dependent. In addition, results of current study revealed gram-positive bacteria were more sensitive than gram negative bacteria to PLA films containing EOs. **CONCLUSIONS:** The results of this study indicated that PLA films containing MP and BP EOs may be useful for packaging of foods in order to increase their shelf life and safety.

Introduction

There is an increasing consumer interest in reducing or replacing non-biodegradable food packaging with biodegradable materials due to environmental pollution caused

by plastic and limitation of petroleum resources (Liu et al., 2007). Antimicrobial film packaging is one kind of biodegradable food packaging that can enhance safety or increase shelf life of foods.

Poly lactic acid (PLA) is a biodegradable polymer made via the polymerization of lactic acid. The monomer, which is also the final degradation product, can be derived through the fermentation of carbohydrate feed stocks. As a packaging material, PLA has good tensile strength comparable to that of petroleum derived thermoplastics and is degradable with good sealed property at low temperatures. Furthermore, PLA is a good water-vapor barrier and is resistant to oil, and has relatively low gas transmittance. PLA also has shown antimicrobial activity in oligomers solution or in combination with organic acids or antimicrobial agents (Liu et al., 2007).

Essential oils are oily, aromatic and liquids that are obtained from different parts of plant material. It has long been recognized that some EOs have antimicrobial properties (Burt, 2004).

Bunium persicum is an economically important medicinal plant growing wild in the dry temperature regions in Iran (Shahsavari et al., 2008). The seeds are rich in essential oil (up to 7%) (Chizzola et al., 2014). In the indigenous system of medicines, seeds are considered as stimulants and antiflatulence and are useful in diarrhea and indigestion. *Bunium persicum* is used for Cuisine purposes such as flavoring foods and beverages (Shahsavari et al., 2008).

Mentha is a genus of Lamiaceae family that is perennial herbs. This aromatic plant has wide distribution in the temperate and sub-temperate regions. The essential oils of peppermint, spearmint, cornmint and bergamotmint are steam distilled from the mint herbage and processed into flavoring for food, medicine, mouthwash, toothwash, chewing gum and confectionery. Peppermint oil is very high-producing high-consumption essential oil (Zeinali et al., 2005).

Cellulose is a strong natural polymer that builds material from long fibrous cells. Its monomers bind together with β(1–4) bands. Cellulose nanofibers are low cost, easily available, biodegradable and easy to recycle (Jebali et al., 2013).

Other positive characteristic of cellulose nano fibres are its low energy consumption during manufacturing, light weight and high-strength. Cellulose has supramolecular structure. Cellulose nano fibers as compared to other commercial fibers have good properties such as high surface area, including a high Young's modulus, very large surface to volume ratio, good mechanical properties, high tensile strength, low coefficient of thermal expansion and formation of highly porous mesh (Kaushik et al., 2010).

The aim of this study was to evaluate antimicrobial activity of PLA films incorporated with different concentrations of BP and MP essential oils (0, 0.5and 1% v/v) and cellulose nano particle (0 and 1% v/v) by disk diffusion method.

Material and Methodes

Plant material and nanoparticle: *Bunium persicum* seeds were collected from June to July 2015, from Kerman, Iran. Dried leaves *Menta piperita* were purchased from Pakan Bazr Esfahan. For extraction of essential oils hydro distillation method was used employing a Clevenger type apparatus for a period of 3h (Aggarwal et al., 2002). Cellulose nanoparticle was purchased from

Noano Novin Polymer Co. Ltd. (Mazandaran, Iran).

Gas Chromatography-Mass Spectrometry: The EO yield of the air-dried material was analyzed by gas chromatography (Kelly, 2000). The chromatograph injector temperature was 250 °C, initial temperature was 50 °C and equipped with DB5 capillary column (30 × 0.25 mm ID ×0.25-mm film thickness) program rate 2.5 °C, final temperature 265 °C. The carrier gas was helium and the split ratio was 1:120. The mass spectrometry (MS) was run in the electron ionization mode, using an ionization energy of 70 eV.

Preparation of PLA film: PLA films were prepared by using solvent casting technique. A solution of PLA was prepared by dissolving the PLA at 1%(w/v) in chloroform and stirring on magnetic stirrer for 8h at room temperature. Then different concentration of *Bunium persicum* and Menta pepperita EOs (0, 0.5 and 1%v/v) and nano cellulose particle (0 and 1% w/v) were added into the solution and stirred by magnetic bar for another 20 min, at room temperature to prepare bioactive films. Finally, the solution was homogenized (Wis 15D, Korea) at 12000 rpm for 2 min and was cast onto glass petri dish with 8 cm diameter and dried over night at room temperature under safety cabinet (Fortunati et al., 2013).

Evaluation of antibacterial activity: Antibacterial activity of films was assessed by using disc diffusion method. Bacterial strains of gram positive (*Staphylococcus aureus* ATCC 65138, *Bacillus cereus* ATCC 11778) and gram negative (*Vibrio parahaemolyticus* ATCC 43996, *Escherichia coli* O157:H7 ATCC 35208 and *Salmonella typhymurium* ATCC 14028) were refreshed twice in sterile brain heart infusion broth at 35 ◦C for 18h (*V. parahaemolyticus* inoculums was prepared by transferring cells from working culture to tube of BHI broth containing 1.5% NaCl). After 18h incubation at 35 ◦C, the second subculture was prepared and incubated for 18h at 35 ◦C. The bacterial broth culture was placed in a 13×100-mm sterile cuvett and optical density (OD) was adjusted to absorbance 0.1, using a spectronic 20 spectrophotometer (Milton Roy Company, Ivyland, PA). Cell concentration inoculated bacteria is about 1×10^7 CFU/ml. The number of cells in the suspension was estimated by duplicate plating from 10-fold serial dilutions on BHI agar and counting the colonies after 18h incubation at 35 oC (Khanjari et al., 2013). Circular sterile discs of PLA films (10 mm diameter containing different concentration of BP, MP essential oils and CN) were placed on Mueller-Hinton agar plates that had previously been inoculated with 100 µl bacterial suspensions$\approx 1\times 10^7$ CFU/ml. These plates were incubated at 37 ◦C for 24h. The experiments were repeated three times. Diameter of inhibition zones was measured by using Digital Caliper and Digimizer software.

Statistical analysis: The effects of EOs concentration on disk halo were evaluated by using Statistical Package for the Social Sciences (SPSS) 19.0 statistical software (SPSS 19.0 for Windows, SPSS Inc., Chicago, IL). One way ANOVA method followed by Tukey tests was used to determine the significant difference. Results were considered statistically significant when $p<0.05$ (Khanjari et al., 2013).

Results

Chemical compositions of the BP and MP essential oils: Gas chromatography–

Table 1. Essential oil composition of *Bunium persicum* identified by GC-MS.

No	RT (min)	Area%	Name
1	6.281	1.54	.ALPHA.-PINENE
2	7.668	0.45	Sabinene
3	7.796	2.95	2-.BETA.-PINENE
4	8.345	0.42	.beta.-Myrcene
5	9.311	0.08	Seudenone
6	9.824	18.23	Cymene
7	9.937	2.78	Limonene
8	10.287	0.19	trans-.beta.-Ocimene
9	11.247	10.48	.gamma.-Terpinene
10	12.407	0.13	.ALPHA.-TERPINOLENE
11	15.309	0.14	L-MENTHONE
12	15.91	0.09	Cymene
13	16.223	0.14	Menthol
14	16.403	0.51	.gamma.-Terpinene
15	16.896	0.10	o-Isopropenyltoluene
16	17.055	0.07	p-Menth-1-en-8-ol
17	17.183	1.09	3-Cyclopentylcyclopentan-1-one
18	19.905	34.08	Propanal, 2-methyl-3-phenyl-
19	21.076	0.45	Isoterpinolene
20	21.266	0.17	.Phellandral
21	21.877	8.07	2-Caren-10-al
22	22.298	12.37	Myrtenal
23	22.883	0.21	Carvacrol
24	25.056	0.41	Benzaldehyde, p-isopropyl
25	27.593	0.70	.Cuminyl acetate
26	28.399	0.17	Cumic acid
27	29.169	0.49	Benzene, butyl-
28	31.038	0.34	Myristicin
29	32.892	0.30	Caryophyllene oxide
30	34.233	0.96	Dillapiole
31	49.921	0.08	n-Pentacosane

mass spectrometry (GC-MS) was used for essential oils analysis. The major components of BP essential oil are presented in Table 1. Based on the findings, a total of 31 constituents were identified which represent 98.17% of the BP EO. The dominant compounds of BP essential oil in our study were Propanal 2-methyl-3-phenyl (34.08%), Cymene (18.23%) and Myrtenal (12.37%). As illustrated in Table 2, a total of 35 constituents were identified in MP essential oil which represent 99.30% of the EO. The dominant compounds of MP essential oil in our study were p-Menthan-3-ol (44.59%), p-Menthan-3-one, trans (12.14%).

Table 2. Essential oil composition of *Mentha piperita* identified by GC-MS.

No	RT (min)	Area%	Name
1	6.281	0.94	.ALPHA.-PINENE
2	7.662	0.37	Sabinene
3	7.775	1.31	2-.BETA.-PINENE
4	8.345	0.18	.beta.-Myrcene
5	8.546	0.12	3-Octanol
6	9.336	0.34	.ALPHA. TERPINENE
7	9.686	0.65	Cymene
8	9.989	9.56	1,8-Cineole
9	10.281	0.08	.beta.-trans-Ocimene
10	11.134	1.76	.gamma.-Terpinene
11	11.478	0.57	.gamma.-Terpinene
12	12.402	0.17	.ALPHA.-TERPINOLENE
13	13.034	0.20	.DELTA.3-Carene
14	13.943	0.10	.beta.-Phellandrene
15	15.976	12.14	p-Menthan-3-one, trans
16	16.808	44.59	p-Menthan-3-ol
17	16.942	0.46	neo-Menthol
18	17.25	1.21	Linalyl propionate
19	19.535	3.15	p-Menth-4(8)-en-3-one
20	20.254	0.84	Piperitone
22	22.37	6.53	cis-Carane
23	22.883	0.80	p-Menth-3-ene
24	25.682	0.08	.alpha.-Copaene
25	26.031	0.54	.BETA. BOURBONENE
26	26.365	0.10	.BETA. ELEMENE
28	27.413	5.60	TRANS(.BETA.)-CARYOPHYLLENE
29	27.716	0.09	GERMACRENE-D
30	28.84	0.72	trans-.beta.-Farnesene
31	29.636	4.46	Germacrene D
32	30.114	0.51	bicyclogermacrene
33	30.396	0.09	germacrene A
34	31.033	0.19	.delta.-Cadinene
35	33.19	0.85	Ledene

Antimicrobial activity: The antibacterial activity of PLA films containing BP and MP essential oils and CN at different concentra-

Table 3. Inhibition effect of *Bunium persicum* EO on different types of bacteria. Same row followed by the same lowercase letter are not significantly different. Same column followed by the same uppercase letter are not significantly different.

	0%EO	0%EO+nano	0.5%EO	0.5%EO+nano	1.5%EO	1.5%EO + nano
Bacillus cereus	0.00±0.00^{Aa}	0.00±0.00^{Aa}	19.26±2.17^{Ab}	18.23±0.27^{Ab}	23.53±0.57^{Ac}	23.16±0.49^{Ac}
Salmonella typhymorium	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}
Staphylococcus aureus	0.00±0.00^{Aa}	0.00±0.00^{Aa}	20.70±0.65^{Ab}	18.25±0.14^{Ac}	23.6±0.55^{Ad}	24.16±0.57^{Ad}
Vibrio parahaemolyticus	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	13.93±0.94^{Cb}	15.79±0.24^{Cb}
E. coli	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}

Table 4. Inhibition effect of *Mentha piperita* EO on different types of bacteria. Same row followed by the same lowercase letter are not significantly different. Same column followed by the same uppercase letter are not significantly different.

	0%EO	0%EO+nano	0.5%EO	0.5%EO+nano	1.5%EO	1.5%EO + nano
Bacillus cereus	0.00±0.00^{Aa}	0.00±0.00^{Aa}	11.25±0.80^{Ab}	16.15±0.74^{Ac}	18.44±1.93^{Ad}	18.25±0.75^{Ad}
Salmonella typhymorium	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ca}	0.00±0.00^{Ba}
Staphylococcus aureus	0.00±0.00^{Aa}	0.00±0.00^{Aa}	12.05±1.46^{Ab}	15.22±1.17^{Ac}	19.47±0.99^{Ad}	19.23±0.86^{Ad}
Vibrio parahaemolyticus	0.00±0.00^{Aa}	0.00±0.00^{Aa}	10.70±0.49^{Ab}	10.47±0.35^{Cb}	12.77±0.31^{Bc}	11.88±0.64^{Cbc}
E. coli	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ca}	0.00±0.00^{Ba}

tions, against tested bacteria are shown in Tables 3 and 4.

Based on our findings, pure PLA films and PLA containing CN did not show any antimicrobial effect. PLA films containing BP essential oil showed an inhibitory effect, at 0.5% concentration, against 2 of the 5 tested bacteria (*Bacillus cereus* 19.26±2.17 cm and *Staphylococcus aureus* 20.70±0.65 cm), whereas PLA films containing the same concentration of MP essential oil showed inhibitory activity against 3 of the 5 of bacteria (*Bacillus cereus* 11.25±0.80 cm, *Staphylococcus aureus* 12.05±1.46 cm and *Vibrio parahaemolyticus* 10.70±0.49 cm). In this concentration, *Bacillus cereus* was the most sensitive bacteria. At 1.5% concentration, BP and MP essential oil showed an inhibitory effect against 3 of the 5 of bacteria (*Bacillus cereus* 23.53±0.57 cm and 18.44±1.93 cm, *Staphylococcus aureus* 23.6±0.55 cm and 19.47±0.99 cm and *Vibrio parahaemolyticus* 13.93±0.94 cm and 12.77±0.31 cm in BP and MP EOs , respectively) but BP EO was more effective than MP EO at 1.5% concentration and *Staphylococcus aureus* was more sensitive than *bacillus cereus* and *Vibrio parahaemolyticus*.

Discussion

Chemical compositions of the BP and MP essential oils: According to the results of this study, major dominant compounds of BP essential oil were Propanal 2-methyl-3-phenyl (34.08%), Cymene (18.23%) and Myrtenal (12.37%). This was in line with the findings of Taherkhani et al. (2015) who identified 21 constituents which accounted for 98.97% of the EO and a major constituent of BP essential oil in their study was Propanal 2-methyl-3-phenyl and gamma-terpinene. However, contradictory findings were reported by Chizzola et al. (2014) and Pourmortazavi et al. (2005), who noted that gamma-terpinene, cuminaldehyde, a-methyl-benzenemethanol were the major compounds of BP essential oil extracted from Iran (Taherkhani et al., 2015, Pourmortazavi et al., 2005, Chizzola et al., 2014).

As previously described, the main compounds of MP essential oil in current study were p-Menthan-3-ol (44.59%), p-Menthan-

3-one, trans (12.14%). That it's not according to GOKalp et al. (2002) who noted that the major components of MP essential oil were menthol (28-42%) and menthone (18-28%) (11) and GC–MS analysis in Yadegarinia et al. (2006) study determined that MP contained a-terpinene (19.7%), isomenthone (10.3%), trans-carveol (14.5%), pipertitinone oxide (19.3%) (Yadegarinia et al., 2006).

The reasons for the differences in the chemical composition of essential oils are different factors such as species, age of the plant, plant part used for extraction, geographic area, soil, weather conditions, harvest season, extraction technique, etc. (Ghnaya et al., 2013, Chamorro et al., 2012).

Antimicrobial activity: The antibacterial activity of PLA films containing BP and MP essential oils and CN at different concentrations, against tested bacteria are shown in Tables 3 and 4. Results of our study illustrated pure PLA film did not show any antimicrobial activity against all of the five tested bacteria ($p>0.05$). Similar findings are reported by Erdohan et al. (2013), Jin and Zhang (2008), Jin (2010) and Liu et al. (2007), Llana-Ruiz-Cabello et al. (2016), who noted no significant antimicrobial activity for the pure PLA film (Jin and Zhang, 2008, Jin, 2010 , Liu et al., 2007, Erdohan et al., 2013, Llana-Ruiz-Cabello et al., 2016). Pure PLA film did not show any antimicrobial activity maybe because of the hydrophobic nature of PLA that limited microorganism access to PLA film (Rhim et al., 2009). Results of this study revealed, PLA films containing BP essential oil showed an inhibitory effect, at 0.5% concentration, against 2 of the 5 tested bacteria (*Bacillus cereus* and *Staphylococcus aureus*), whereas PLA films containing the same concentration of MP essential oil showed inhibitory activity against 3 of the 5 of bacteria (*Bacillus cereus*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*). At 1.5% concentration, BP and MP essential oil showed an inhibitory effect against 3 of the 5 bacteria (*Bacillus cereus*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*), however BP is more effective than MP EOs at 1.5%concentration. Also results of current study revealed a concentration-dependence for inhibitory activity of films ($p<0.05$). Antimicrobial effect of EOs could be due to volatile compounds in EOs. Stronger inhibition effect of BP EO can be for more methyl compounds in BP EO (Siddique et al., 2017).

Similar finding is reported by Erdohan et al. (2013) who noted increasing of the olive leaf extract in the film discs from 0.9 mg to 5.4 mg resulted in a significant increase in inhibitory zones from 9.10 mm to 16.20 mm, respectively (Erdohan., 2013). Pranoto et al. (2005) declared chitosan and alginate edible based films incorporated by garlic oil did not show inhibitory activity against *Escherichia coli* and *Salmonella typhimurium* (Pranoto and Salokhe, 2005, Pranoto and Rakshit 2005). Results of Llana-Ruiz-Cabello et al.'s (2016) study revealed PLA films incorporated with oregano essential oil (2, 5 and 10%) had significant antimicrobial activity against *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Salmonella enteritica*, *Staphylococcus carnosus* and *Escherichia coli* O157:H7. In addition, their results revealed that gram positive bacteria were more sensitive than gram negative bacteria (Llana-Ruiz-Cabello, 2016). These patterns are in accordance with our current study that shows none of our EOs or their combinations were effective against E.coli and Salmonella enterica. This may be due

to outer membrane structure in cell wall of gram-negative bacteria that contains hydrophilic lipopolysaccharide (Denyer and Maillard, 2002). However, contradictory findings were reported by Shakeri et al. (2011) who noted that WPI films incorporated with 2% (v/v) *Zataria multiflora* essential oil inhibited the growth of all pathogenic bacteria and gram negative bacteria were more sensitive than gram-positive bacteria (Shakeri et al., 2011).

Our findings, similar to previous studies, have shown that PLA films containing nano cellulose did not show any antimicrobial activity against foodborne pathogens used in this study. Also, adding nano cellulose to PLA- EO composite decreased antimicrobial activity of PLA films. Salmieri et al. (2014) and Boumail et al. (2013), López et al. (2007) noted molecular interactions between EO and PLA–CN crystals matrix could influence the diffusion rate of EO volatiles via hydrophobic interactions from EO components (Salmieri et al., 2014, Boumail et al., 2013, López et al., 2007).

According to the results of this study PLA films containing *Bunium persicum* and *Mentha piperita* essential oils and cellulose nanoparticles have significant antibacterial effect at concentrations of 0.5% and higher on gram positive bacteria. Because of environmental problems caused by oil-based material packaging and in order to decrease the food borne diseases, use of PLA films incorporated with *bunium persicum* and *Mentha piperita* essential oils and cellulose nanoparticle for food packaging may be useful.

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ارزیابی اثر ضدمیکروبی فیلم‌های پلی لاکتیك اسید حاوی نانوسلولز و اسانس‌های زیره سیاه و نعناع فلفلی

فاضله طالبی علی میثاقی* علی خنجری ابوالفضل کامکار حسن گندمی معصومه سعیدی

گروه بهداشت و کنترل مواد غذایی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

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چکیده

زمینه مطالعه: دراین مطالعه اثر ضدمیکروبی فیلم‌های پلی لاکتیك اسید حاوی نانوسلولز، اسانس‌های زیره سیاه و نعناع فلفلی بر روی برخی از باکتری‌های پاتوژن مورد مطالعه قرار گرفت. **هدف:** هدف از این مطالعه دست‌یابی به یك بسته بندی دوستار محیط زیست با افزایش ایمنی مواد غذایی بود. **روش کار:** بعد از اسانس‌گیری ترکیبات شیمیایی اسانس‌ها توسط GC-MS آنالیز گردید. فیلم‌های پلی لاکتیك اسید حاوی غلظت‌های مختلف اسانس‌های زیره سیاه و نعناع فلفلی (۰، ۰/۵ و ۱٪) و نانو سلولز (۰ و ۱٪) به روش قالب گیری تهیه گردید و اثر ضد میکروبی آنها بر علیه باکتری‌های گرم مثبت (استافیلوکوکوس اورئوس و باسیلوس سرئوس) و باکتری‌های گرم منفی (ویبریوپاراهمولیتیکوس، اشرشیاکلی، سالمونلا تیفی موریوم) به روش انتشار دیسك مورد ارزیابی قرار کرفت. **نتایج:** ترکیبات اصلی اسانس زیره سیاه شامل پروپانال۲-متیل-۳-فنیل (۳۴/۰۸٪)، سیمن (۱۸/۲۳٪) و میرتنال (۱۲/۳۷٪) می‌باشد. همچنین پی-منتال-۳-اول (۴۴/۵۹٪) و پی-منتان-۳-وان، ترنس (۱۲/۱۴٪) عمده ترکیبات اسانس نعناع فلفلی را تشکیل می‌دهند. نتایج این مطالعه نشان داد که فیلم خالص پلی لاکتیك اسید و یا همراه با نانوسلولز هیچ گونه اثر ضد میکروبی بر روی پنج باکتری مورد مطالعه نداشت اما فیلم های حاوی اسانس ها اثر ضد میکروبی معنی داری را نشان دادند ($p<0/05$) که این اثر در فیلم ترکیب شده با اسانس زیره سیاه قوری تر از اسانس نعناع فلفلی می‌باشد. همچنین اثر مهاری فیلم ها وابسته به غلظت بود به علاوه نتایج این مطالعه آشکار ساخت باکتری‌های گرم مثبت نسبت به باکتری های گرم منفی حساسیت بالاتری نسبت به فیلم های پلی لاکتیك اسید حاوی اسانس داشتند. **نتیجه گیری نهایی:** نتایج این مطالعه آشکار ساخت که فیلم‌های پلی لاکتیك اسید حاوی اسانس زیره و نعناع فلفلی ممکن است برای بسته بندی مواد غذایی به منظور افزایش زمان نگهداری و ایمنی آنها مفید واقع شود.

واژه های کلیدی: زیره سیاه، نانوذرات سلولز، انتشار دیسک، نعناع فلفلی، پلی لاکتیك اسید

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*) نویسنده مسؤول: تلفن: ۶۱۱۱۷۰۴۰ (۲۱)۹۸+ نمابر: ۶۶۴۳۸۱۴۱ (۲۱)۹۸+ Email: amisaghi@ut.ac.ir