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Study of the antimicrobial effect of a polyvinyl alcohol-carboxymethyl cellulose and quince seed mucilage composite film containing *Perovskia* essential oil

Ghasemloo, M¹., Misaghi, A^{2*}., Abolfazl Kamkar²., Hassan Gandomi Nasrabadi²., Ali Khanjari²

1- Ph.D. student, Department of Food Hygiene, University of Tehran, Faculty of Veterinary Medicine, Tehran, Iran.

2- Department of Food Hygiene, University of Tehran, Faculty of Veterinary Medicine, Tehran, Iran

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*Corresponding Author E-

ABSTRACT

The use of biodegradable films as a modern technology in addition to being environmentally friendly and non-toxic, protects food from physical, chemical, and biological damage. Biodegradable films act as carriers of antioxidant and antimicrobial compounds. In this study, the antimicrobial effect of polyvinyl alcohol-carboxymethyl cellulose and quince seed mucilage biodegradable film containing *Perovskia* essential oil on four species of the most important foodborne pathogenic bacteria including *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* (O157:H7), and *Salmonella Typhimurium* was investigated using the Disk Diffusion Assay method. From films produced based on polyvinyl alcohol-carboxymethyl cellulose and quince seed mucilage containing concentrations of 0.25, 0.5, and 1% of *Perovskia* essential oil, discs with a diameter of 10 mm were prepared and placed under sterile conditions on previously cultured Mueller-Hinton medium. The diameter of the formed halos was considered as an indicator of the antimicrobial activity of the films. Statistical calculations were performed using SPSS Ver.22 Software. Assessment of the antibacterial activity of the active films on gram-positive and gram-negative bacteria indicated that the highest antibacterial activity was related to the treatments polyvinyl alcohol-carboxymethyl cellulose + 1% quince seed mucilage + 1% *Perovskia* essential oil and polyvinyl alcohol-carboxymethyl cellulose + 1% *Perovskia* essential oil respectively. In addition, according to the results of current study *Staphylococcus aureus* revealed the highest (4.57 mm) and *Salmonella Typhimurium* the lowest sensitivity (3.23 mm) to the prepared films. The results show that the *Perovskia* essential oil can be used as a natural preservative in the composition of polyvinyl alcohol-carboxymethyl cellulose and seed mucilage biodegradable film, for food packaging.

1- INTRODUCTION

In recent years, the adoption of advanced technologies aimed at improving the safety and overall quality of food products has gained substantial significance [1]. One of the main factors in ensuring the safety of food products is their packaging, as it protects the product against physical, microbial, and chemical hazards, thereby playing an important role in safeguarding consumer health and extending shelf life [2]. With the growing preference of consumers for high-quality and fresh food products, along with their increasing concerns regarding the use of synthetic preservatives, the development of biodegradable edible coatings and films endowed with strong antimicrobial and antioxidant properties has attracted considerable attention as a safe and promising alternative for both researchers and the food industry [3]. As a modern food preservation approach, the use of edible coatings provides several advantages such as being ingestible, environmentally compatible, non-toxic, and cost-effective while also protecting food products from physical, chemical, and biological damage. Furthermore, these coatings serve as an effective barrier that regulates the exchange of gases and moisture and limits the penetration of microorganisms [4]. Additionally, edible coatings can serve as carriers for food additives, helping to maintain the quality and extend the shelf life of food products from the point of production to the moment they reach the consumer [5]. Biodegradable coatings can be prepared from a variety of natural materials, including lipids, polysaccharides, proteins, and their derivatives [6]. Among the various types of edible coatings, cellulose and its derivatives have received considerable attention, particularly over the past decade, due to their outstanding environmental attributes, including biodegradability, edibility, and non-toxicity. Due to its unique chemical structure, cellulose is a highly crystalline material that is insoluble in water. In contrast, carboxymethyl cellulose (CMC), a derivative of cellulose, is an anionic polysaccharide produced by the substitution of hydroxyl groups with carboxymethyl groups [7]. It is also considered one of the important and cost-effective polysaccharides, produced through the

reaction of cellulose with sodium hydroxide and monochloroacetic acid [8]. This compound is a linear polymer with a relatively high molecular weight, capable of dissolving in both cold and hot water, and can independently form strong and durable films [9]. This biopolymer possesses excellent film-forming properties, low cost, and unique physical characteristics, including water solubility, viscosity, thermoplastic behavior, and resistance to biodegradation [10]. Quince seed mucilage consists of cellulose and polysaccharide compounds that can be readily hydrolyzed. Due to its ease of extraction, this material has attracted attention as a potential candidate for film production or incorporation into edible coating formulations. The health-promoting properties of quince seed mucilage include its potential to induce the destruction of cancer cells [11], aid in digestive problems [12] and stimulate growth factors as well as accelerate skin healing [13]. Polyvinyl alcohol (PVA) is a synthetic, non-toxic, thermoplastic, semi-crystalline polymer of fossil origin, obtained through the hydrolysis of polyvinyl acetate, and is considered one of the most versatile synthetic polymers [14]. It is also one of the rare polymers that, despite containing only carbon-carbon single bonds, is readily biodegradable. Its notable features include a high film-forming capability, suitable tensile strength, and effective performance in preventing the permeation of gases and aromatic compounds [15]. The control of pathogenic microorganisms in food products requires the use of chemical antimicrobial agents. Among the major foodborne pathogenic microorganisms are *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* (O157:H7), and *Salmonella Typhimurium*, which annually cause foodborne illnesses in developing communities [16]. The application of natural antimicrobial agents, such as essential oils, plays a significant role in extending the shelf life of food products by inhibiting chemical processes, including oxidation, and by exhibiting antimicrobial activity [17]. *Brazambel* (known locally as Gol Kabood, Domo, and Gore), with the scientific name *Proveskia abrotanoides* Karel, is a small genus belonging to the Lamiaceae family. It is a perennial shrub with blue to purplish flowers and is classified within the mint family [18,19]. Plants of the Lamiaceae family contain

important compounds such as terpenoids, flavonoids, as well as phenolic compounds, fatty acids, steroidal constituents, and glycosidic iridoids. The flavonoids, phenolic compounds, and anthocyanins present in the flowering shoots of *P. abrotanoides*, due to the abundance of these bioactive constituents, exhibit strong antioxidant, anti-inflammatory, and analgesic properties [20]. Some of the antimicrobial compounds extracted from this plant include 1,8-cineole (32.4%), myrcene (13%), α -pinene (10.2%), camphor (9.1%), β -caryophyllene (7.9%), α -humulene (6.4%), camphene (5%), and α -bisabolol (2.6%), which have wide applications in the pharmaceutical, cosmetic, and food industries [21]. In traditional medicine, it has been used for the treatment of cardiovascular diseases, liver disorders, infectious diseases, leishmaniasis, rheumatic pains, and various inflammatory conditions [22]. Edible films enriched with antimicrobial compounds have been extensively studied due to their ability to extend the shelf life of food products and their inhibitory effects against foodborne pathogens, particularly *Listeria monocytogenes* and *Escherichia coli* O157:H7 [23]. The aim of this study was to develop an antimicrobial film containing *Proveskia abrotanoides* Karel essential oil and to evaluate its antimicrobial effects against selected foodborne pathogens, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella Typhimurium*, using the disk diffusion method.

2-Materials and Methods

2.1. Materials

Proveskia abrotanoides Karel plants were collected from the northern regions of Khorasan Razavi Province and identified by the Plant Sciences Research Institute of Ferdowsi University, Mashhad. Polyvinyl alcohol (molecular weight 89,000–98,000, 99% hydrolyzed), carboxymethyl cellulose (molecular weight 250,000 g/mol, high viscosity, CP 1500–3000), acetic acid, Whatman No. 42 filter paper, glycerol (87% purity) as a plasticizer, calcium chloride as a crosslinking agent, and Tween 80 as an emulsifier were obtained from Merck, Germany. The microbial strains used in this study were obtained from the Culture Collection of the Iranian Research Organization for Science and Technology. It should be noted

that all culture media, chemical solutions, and consumables used in this study were purchased from Merck, Germany, and the culture media were prepared according to the manufacturer's instructions. To complete different experimental procedures in this study, a IKA T25-Digital homogenizer (Itra Turrax, Germany) and a digital caliper with an accuracy of ± 2 mm (Scientific Fisher) were used.

2.2. Essential Oil Extraction

Proveskia abrotanoides Karel plants were prepared, and after identification, essential oil extraction was done using a Clevenger apparatus. In each batch, 100 g of *Proveskia abrotanoides* Karel powder was mixed with distilled water at a 1:10 ratio and subjected to hydrodistillation for 3 hours using the Clevenger apparatus. The extracted essential oil was collected in vials and stored at 4 °C in the dark until further use [24].

2.3. Preparation of Films

For preparation of composite films of polyvinyl alcohol–carboxymethyl cellulose and quince seed mucilage containing *Proveskia abrotanoides* Karel essential oil were prepared using the solution casting method was used. Polyvinyl alcohol solution 2% (w/v), carboxymethyl cellulose 1% (w/v), and quince seed mucilage 1% were dissolved in water, then 0.5% (v/v) glycerol as a plasticizer was added. Tween 80 (0.25% solution) was added as an emulsifier, along with the desired concentrations of *Proveskia abrotanoides* Karel essential oil (0.25%, 0.5%, and 1% v/v), and the mixture was homogenized at 12,000 rpm for 2 minutes. The resulting solution was poured into glass plate (8–10 cm in diameter). After the films were dried at room temperature, they were carefully removed from the plates and used for experimental procedures [25].

2.4. Activation of Standard Microbial Strains

The antibacterial effects of the prepared films were evaluated against four common foodborne pathogens. The pathogenic bacteria used in this study were *Listeria monocytogenes* (ATCC 19111), *Escherichia coli* O157:H7 (ATCC 10536), *Staphylococcus aureus* (ATCC 25923), and *Salmonella Typhimurium* (ATCC 14028). To activate the microbial strains, the stock cultures were inoculated onto brain heart

infusion (BHI) agar and incubated at 37 °C for 24 hours. For the preparation of the inoculum, bacterial cells were transferred to tubes containing brain heart infusion (BHI) broth and incubated at 35 °C for 18 hours. The resulting microbial suspension was then transferred into sterile cuvettes to achieve a bacterial density of 1×10^8 colony-forming units (CFU)/mL using a spectrophotometer at a wavelength of 600 nm. The number of bacterial cells in the suspensions were further estimated by plating on BHI agar [26].

2.5. Assessment of the Antimicrobial Effect of Polyvinyl Alcohol–Carboxymethyl Cellulose and Quince Seed Mucilage Composite Films Containing *Proveskia abrotanoides* Karel Essential Oil on Bacteria

The antimicrobial activity of the polyvinyl alcohol–carboxymethyl cellulose and quince seed mucilage composite films containing different concentrations of *Proveskia abrotanoides* Karel essential oil was assessed under laboratory conditions using the disk diffusion method [27]. Under sterile conditions and in safety cabinet class II, 100 µL of bacterial suspensions at a concentration of 1×10^7 CFU/mL were transferred onto Mueller–Hinton agar with a pipette and evenly spread over the surface of the medium. The films containing different concentrations of *Proveskia abrotanoides* Karel essential oil, punched into 10 mm diameter disks, were placed onto the agar surface. The plates with the disks were incubated at 37 °C for 24 hours, then they were removed and examined. The diameter of the inhibition zones formed around the disks was measured and recorded using a caliper. Films of polyvinyl alcohol and carboxymethyl cellulose without essential oil used as controls.

2.6 .Statistical Analysis

This study was done as a completely randomized design with three replicates. The experimental treatments included eight treatments tested against four foodborne

pathogenic bacteria. To assess the significance of the effects of different essential oil concentrations on bacterial growth inhibition, SPSS version 22 and SigmaPlot software were used. One-way analysis of variance (ANOVA) was also used to compare the means, and differences were considered statistically significant at $P < 0.05$.

3-Results

3.1. Antimicrobial Effect of Polyvinyl Alcohol–Carboxymethyl Cellulose and Quince Seed Mucilage Composite Films Containing Different Concentrations of *Proveskia abrotanoides* Karel Essential Oil

The results of the mean of antimicrobial activity, the diameter of the inhibition zones, of the active films against Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella Typhimurium*) are presented in Table 1. According to the results, the different treatments had a significant effect on the antimicrobial activity of the active films ($P < 0.05$). As shown in Table 1, the control films exhibited no antimicrobial activity ($P > 0.05$). While the addition of quince seed mucilage and *Proveskia abrotanoides* Karel essential oil significantly enhanced the antimicrobial activity of the active films ($P < 0.05$). Furthermore, increasing the essential oil concentration from 0.25% to 1% resulted in a significant improvement in antimicrobial activity ($P < 0.05$). Among the treatments, the highest antimicrobial activity was observed in T7 and T3 (control + 1% quince seed mucilage + 1% *P. abrotanoides* Karel essential oil and control + 1% *P. abrotanoides* Karel essential oil, respectively) ($P < 0.05$). Moreover, the greatest antimicrobial effect of the active films was observed against *Staphylococcus aureus*, a Gram-positive bacterium ($P < 0.05$).

Table 1- The mean results of the inhibition zone diameter (in mm) of active films against selected pathogens

SalmonellaTyphimurium	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i>	Treatments
0/00 ± 0/00 ^f	0/00 ± 0/00 ^f	0/00 ± 0/00 ^h	0/00 ± 0/00 ^g	Co
1/40 ± 0/10 ^c	2/50 ± 0/10 ^f	1/80 ± 0/10 ^f	2/33 ± 0/12 ^f	T1

1/77 ± 0/12 ^d	3/00 ± 0/10 ^d	2/47 ± 0/06 ^d	2/70 ± 0/10 ^d	T2
2/83 ± 0/06 ^b	3/63 ± 0/06 ^b	3/37 ± 0/06 ^b	3/30 ± 0/10 ^b	T3
0/07 ± 0/06 ^f	0/13 ± 0/06 ^g	0/13 ± 0/06 ^g	0/10 ± 0/00 ^g	T4
1/80 ± 0/10 ^d	2/83 ± 0/06 ^c	1/97 ± 0/06 ^c	2/57 ± 0/06 ^c	T5
2/30 ± 0/10 ^c	3/40 ± 0/10 ^c	2/63 ± 0/12 ^c	2/97 ± 0/06 ^c	T6
3/23 ± 0/06 ^a	4/57 ± 0/06 ^a	3/57 ± 0/06 ^a	3/53 ± 0/06 ^a	T7

Co: Control (PVA/CMC); T1: Control + 0.25% *Proveskia* EO; T2: Control + 0.5% *Proveskia* EO; T3: Control + 1% *Proveskia* EO; T4: Control + 1% Quince seed mucilage; T5: Control + 1% Quince seed mucilage + 0.25% *Proveskia* EO; T6: Control + 1% Quince seed mucilage + 0.5% *Proveskia* EO; T7: Control + 1% Quince seed mucilage + 1% *Proveskia* EO.

Different lowercase letters indicate significant differences in each column ($p < 0.05$).

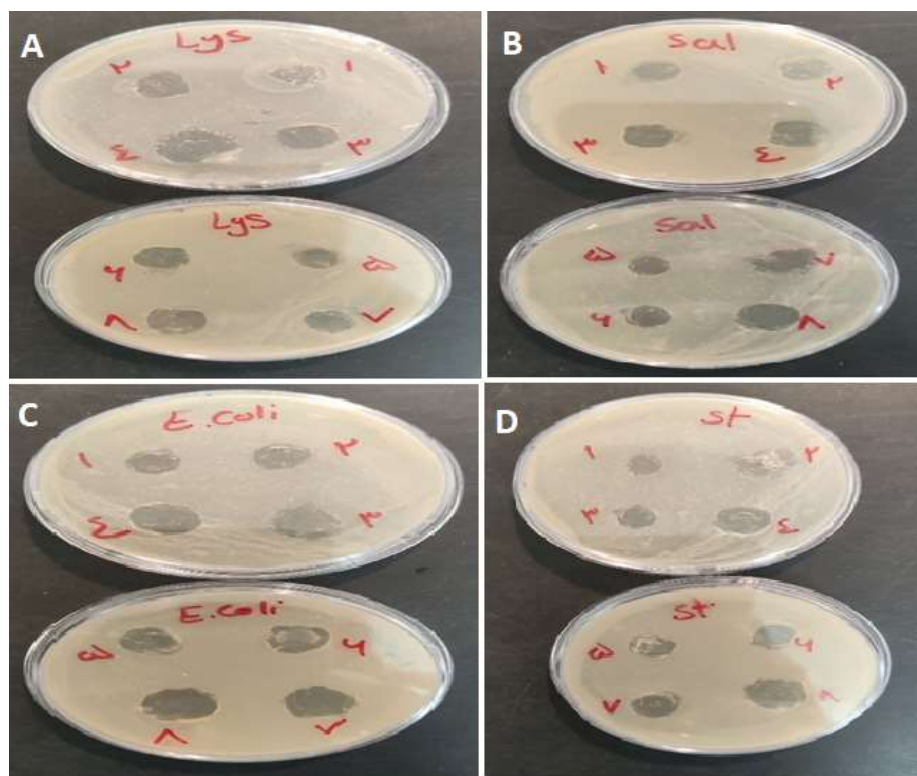


Figure 1. Evaluation of the antibacterial activity of polyvinyl alcohol–carboxymethyl cellulose (PVA/CMC) composite films incorporated with quince seed mucilage and various concentrations of *Proveskia* essential oil against four pathogenic bacteria.

Tested bacteria: A: *Listeria monocytogenes* / B: *Salmonella Typhimurium* / C: *Escherichia coli* O157:H7 / D: *Staphylococcus aureus*

Treatments: 1. PVA/CMC/ 2. PVA/CMC + 0.25% *Proveskia* essential oil/ 3. PVA/CMC + 0.5% *Proveskia* essential oil /4. PVA/CMC + 1% *Proveskia* essential oil / 5. PVA/CMC + 1% quince seed mucilage/ 6. PVA/CMC + 1% quince seed mucilage + 0.25% *Proveskia* essential oil/ 7. PVA/CMC + 1% quince seed mucilage + 0.5% *Proveskia* essential oil/ 8. PVA/CMC + 1% quince seed mucilage + 1% *Proveskia* essential oil

4- Discussion

Scientific studies have indicated that active packaging technology incorporating antimicrobial compounds can inhibit microbial growth within the packaging and enhance the microbial stability of products during storage [28]. The results of the mean antimicrobial activity, the diameter of the inhibition zones, of the active film samples against Gram-positive bacteria (*Staphylococcus aureus* and *Listeria*

monocytogenes) and Gram-negative bacteria (*Escherichia coli* and *Salmonella Typhimurium*) are presented in Table 1. The control films (polyvinyl alcohol–carboxymethyl cellulose) exhibited no antimicrobial activity against the tested Gram-positive and Gram-negative bacteria. While, the incorporation of quince seed mucilage significantly enhanced the antimicrobial activity of the active films. This can be due to the presence of phenolic acids, antioxidants, and flavonoids in the mucilage [29].

Furthermore, the results indicated that the incorporation of *Proveskia abrotanoides* Karel essential oil, and also increasing its concentration from 0.25% to 1%, significantly enhanced the antimicrobial activity of the active films. Among the treatments, the highest antimicrobial activity was observed in T7 against the pathogens *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella Typhimurium*, with inhibition zone diameters of 4.57, 3.57, 3.53, and 3.23 mm, respectively. In addition, the greatest antimicrobial effect of the active films was observed against Gram-positive bacteria. The functional mechanisms of essential oils and plant extracts are related to their chemical composition and antimicrobial activity; however, they do not all have similar mechanisms. Nevertheless, most studies have confirmed their effects on the microbial cell wall. The hydrophobic nature of essential oils and plant extracts facilitates their penetration into the lipid bilayer of the cell membrane, increasing its permeability. This disruption interferes with vital membrane-dependent activities, leading to leakage of ions and essential cellular components, finally resulting in cell death. The toxic effects of essential oils and plant extracts on membrane structure and function explain their antimicrobial properties, which are largely attributed to the presence of monoterpenoid compounds [30]. α -Pinene, camphene, β -pinene, and 1,8-cineole have been identified as the major antimicrobial compounds in the essential oil of *Proveskia abrotanoides* Karel [31]. In recent years, the antimicrobial effects of plant essential oils against food-spoilage and pathogenic bacteria, including both Gram-negative and Gram-positive species, have been extensively investigated. Gram-negative bacteria are more resistant to plant essential oils compared to Gram-positive bacteria and this is due to the structure of their cell walls. Approximately 90–95% of the cell wall of Gram-positive bacteria is composed of peptidoglycan, whereas the cell wall of Gram-negative bacteria is more complex, consisting of a thinner peptidoglycan layer and an outer membrane composed of a phospholipid bilayer. Plant essential oil molecules can hardly penetrate the complex cell membrane of Gram-negative bacteria but they easily enter Gram-positive bacteria [32]. Overall, the antimicrobial potential of essential

oils is determined by their chemical composition. Plant essential oils consist of complex mixtures of volatile compounds, with the main active antimicrobial constituents including terpenes such as monoterpenes, sesquiterpenes, diterpenes, and also phenolic compounds [33]. The antimicrobial activity of plant extracts is influenced by their composition, concentration, chemical structures, and functional groups. According to previous studies, the antimicrobial effects of plant extracts and essential oils occur through mechanisms such as cell wall damage, disruption of cell membrane structure, and interference with energy metabolism and enzymatic functions. The lipophilic nature of these plant compounds allows them to easily pass the cell wall and cytoplasmic membrane, as a result having toxic effects on microbial cells [34]. Hydrophobic molecules can easily penetrate the cell walls of Gram-positive bacteria. In contrast, in gram-negative bacteria, the presence of lipopolysaccharides in the outer membrane imparts a hydrophilic character to their cell surface. This structural feature acts as a barrier to the penetration of hydrophobic compounds, resulting in increasing the resistance of this group of bacteria to the antibacterial effects of plant essential oils [35]. On the other hand, phenolic compounds present in plant extracts, such as thymol, eugenol, and carvacrol, can disrupt the outer membrane of the bacterial cell wall. Specifically, the combination of carvacrol with fatty acid chains can lead to membrane expansion and instability, increasing its fluidity and permeability, which finally results in the leakage of intracellular contents [36]. Askarova et al. (2024) demonstrated that the incorporation of *Proveskia abrotanoides* Karel essential oil nanoemulsions into a chitosan polymer matrix enhanced antimicrobial activity against *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa* [37]. The results of the present study also indicated that the incorporation of *Proveskia abrotanoides* Karel essential oil into polyvinyl alcohol–carboxymethyl cellulose–quince seed mucilage composite films resulted in antimicrobial activity, and the antimicrobial activity was increased with the higher concentrations of the essential oil. Mohammad Hosseini et al. (2021) investigated the antimicrobial activity of *Proveskia abrotanoides* Karel essential oil and

reported strong inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*, with inhibition zone diameters of 26.14, 23.74, and 21.29 mm, respectively. Moderate activity was also observed against *Bacillus cereus* and *Pseudomonas aeruginosa*, with inhibition zones of 15.43 mm and 18.86 mm, respectively. Notably, *Bacillus cereus* exhibited higher sensitivity to the essential oil than to the standard antibiotics gentamicin and tetracycline, which were used as positive controls [15]. Noshad (2024) evaluated the antibacterial activity of *Proveskia abrotanoides* Karel essential oil and reported that its antimicrobial effect depended on the bacterial species. *S. aureus* and *S. typhi* were identified as the most sensitive and the most resistant strains to the essential oil, respectively [38]. In the present study, *Staphylococcus aureus* was the most sensitive bacterium, with an average inhibition zone diameter of 4.57 mm, whereas *Salmonella Typhimurium* was the most resistant, with an average diameter of 3.23 mm. Furthermore, the results demonstrated that increasing the concentration of *Proveskia abrotanoides* Karel essential oil enhanced the antimicrobial activity of the films. Muppalla et al. (2014) reported that carboxymethyl cellulose–polyvinyl alcohol films incorporated with garlic essential oil effectively delayed the growth of pathogenic bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and spoilage microorganisms (*psychrotrophic bacteria* and *Pseudomonas species*) in minced chicken meat for up to 12 days [39].

5- Conclusion

In this study, among the designed films, the polyvinyl alcohol–carboxymethyl cellulose films incorporated with 1% quince seed mucilage and 1% *Proveskia abrotanoides* Karel essential oil, as well as those with 1% essential oil alone, demonstrated significant potential in inhibiting the growth of foodborne pathogenic bacteria. Therefore, *Proveskia abrotanoides* Karel can be considered a suitable natural preservative for food packaging to inhibit the growth of foodborne pathogens. Therefore, the findings of the present study may contribute to enhancing the safety and quality of food products.

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Author Contributions

All aspects of this study were carried out by the author.

Conflict of Interests

The author declares that there are no financial conflicts of interest or competing interests related to this study.

Data Availability

The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AI statement

Artificial intelligence tools were used solely for language editing and improving the clarity, writing, and grammar of the manuscript, accounting for approximately 25–30% of the manuscript preparation. The scientific content, interpretation of the results, and final version of the manuscript were developed, reviewed, and approved by the authors.

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مطالعه اثر ضد میکروبی فیلم ترکیبی پلی ونیل الکل-کربوکسی متیل سلولز و موسیلاژ دانه به حاوی

اسانس برازمیل

مرجان قاسملو^۱، علی میثاقی^{۲*}، ابوالفضل کامکار^۲، حسن گندمی نصرآبادی^۲، علی خنجری^۲

۱- دانشجوی دکتری تخصصی گروه بهداشت مواد غذایی، دانشگاه تهران، دانشکده دامپزشکی، تهران، ایران.

۲- گروه بهداشت مواد غذایی، دانشگاه تهران، دانشکده دامپزشکی، تهران، ایران.

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استفاده از پوشش های زیست تخریب پذیر به عنوان یک فناوری نوین، علاوه بر اینکه با محیط زیست سازگار و غیرسمی هستند، قادرند از مواد غذایی در برابر آسیب های فیزیکی، شیمیایی و بیولوژیکی محافظت کنند. فیلم های زیست تخریب پذیر همچنین می توانند به عنوان حامل ترکیبات آنتی اکسیدانی و ضد میکروبی عمل کنند و نقش محافظتی خود را ایفا نمایند. در این مطالعه اثر ضد میکروبی فیلم زیست تخریب پذیر پلی ونیل الکل-کربوکسی متیل سلولز و موسیلاژ دانه به حاوی اسانس برازمیل بر چهار گونه از مهمترین باکتری های پاتوژن غذازاد شامل *استافیلوکوکوس اورئوس*، *لیستریا مونوسیتوژنز*، *اشرشیاکلی (O157:H7)* و *سالمونلا تیفی* موریم با روش آزمون انتشار دیسک (Disk Diffusion Assay) مورد بررسی قرار گرفت. از فیلم های تولید شده بر پایه پلی ونیل الکل-کربوکسی متیل سلولز و موسیلاژ دانه به حاوی غلظت های ۰/۲۵، ۰/۵ و ۱ درصد از اسانس برازمیل دیسک هایی به قطر ۱۰ میلی متر تهیه و در شرایط استریل روی محیط مولر هیتون از قبل کشت داده شده قرار گرفتند. محاسبات آماری با استفاده از نرم افزار SPSS Ver.22 انجام پذیرفت. بررسی فعالیت ضد میکروبی فیلم های فعال بر باکتری های گرم مثبت و منفی نشان داد که بیشترین فعالیت ضد میکروبی به ترتیب در تیمارهای پلی ونیل الکل-کربوکسی متیل سلولز + ۱ درصد موسیلاژ دانه به + ۱ درصد اسانس برازمیل و پلی ونیل الکل-کربوکسی متیل سلولز + ۱ درصد اسانس برازمیل بدست آمد. همچنین بیشترین تأثیر ضد میکروبی بر روی باکتری گرم مثبت *استافیلوکوکوس اورئوس* با هاله عدم رشد ۴/۵۷ میلی متر و کمترین تأثیر ضد میکروبی بر علیه باکتری *سالمونلا تیفی* موریم با هاله عدم رشد ۳/۲۳ میلی متر مشاهده شد. نتایج حاصله نشان می دهند که اسانس روغنی برازمیل می تواند به عنوان نگهدارنده ای طبیعی در ترکیب فیلم زیست تخریب پذیر پلی ونیل الکل-کربوکسی متیل سلولز و موسیلاژ دانه به جهت بسته بندی مواد غذایی مورد استفاده قرار گیرد.

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* مسئول مکاتبات:

mkarami@basu.ac.ir