

Antimicrobial effects of edible nano-composite based on bean pod shell gum, nano-TiO₂, and *Mentha pulegium* essential oil

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ABSTRACT

Natural antioxidants in edible coatings can modify the structure and improves the functionality and applicability of the film in food industries. This study was done to determine the antimicrobial effect of nano-composite based on bean pod shell gum (4% w/v), TiO₂ nano-particles (NPs) (1%–2% w/v) and *Mentha pulegium* essential oil (EO) (2%–4% v/v) on five food-borne pathogens in two categories, including Gram positives and three Gram-negatives bacteria. The antimicrobial activity was tested using disk diffusion test. According to the results, Gram-positive bacteria were more susceptible than Gram-negative bacteria. Increasing *M. pulegium* EO and TiO₂ NPs content increased the antimicrobial activity of the edible film based on bean pod shell gum, so that the treatment containing 4% v/v *M. pulegium* EO and 2% w/v TiO₂ NPs led to the highest inhibition zone (11.8–15.2 mm) compared to treatment containing 2% v/v *M. pulegium* EO and 1% w/v TiO₂ NPs with inhibition zone range of 9.8–11.5 mm. In general, TiO₂ NPs and *M. pulegium* EO improved the functional properties, including antimicrobial activity of the edible film based on bean pod shell gum which increases the potential of films to be used for fresh products.

1. INTRODUCTION

Edible films based on polysaccharides have recently been used in food packaging, because of their antimicrobial and antioxidant activities [1]. Adding antimicrobial agents into the food packages has more advantages to direct addition of these agents in foods [2].

For food packaging applications, the main purpose of the antimicrobial agent is to act against microorganisms and enhance the shelf life and maintain the quality and safety of the foods [3]. Edible coatings contain materials which are suitable for consumption and act as a barrier against water vapor, oxygen, moisture, and other factors. Adding active compounds like antioxidants to these films, enhances the functional properties, especially for food preservation. Different research studies have shown the ability of polysaccharide-based coatings carrying different natural antimicrobial agents to maintain quality and

safety of fresh fruits, such as orange [4]. Bean pod with *Vicia faba* scientific name is one of the sources of polysaccharide gums which belongs to the legume family and is an annual herbaceous plant. Raw bean pod contains proteins, lipids, starch, vitamins, and many minerals [5].

In recent years, many studies have been carried out on natural preservatives, such as essential oils (EOs) and plant extracts. The extracts and EOs of medicinal plants and their constituent parts have known antibacterial effects [6].

Mentha (mint or pudina) is a well-known genus for medicinal and aromatic value. This genus has 25–30 species which cultivated in tropical to temperate climates, such as America, Europe, China, Brazil, and India [7]. *Mentha* spp. has been investigated for their EO compositions and biochemical activities. The antimicrobial efficacy of *Mentha* EOs has been found to vary from low to significant which is due to the chemical composition of the essential oil [8].

Nowadays nano-materials are being used in food packages in which they are added into the polymer to extend the gas barrier

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properties or where the main role of nano-particles (NPs) is for better protection of the food, such as TiO₂ and silver NPs, as potent antimicrobial agents [9]. Emerging metal NPs with biocide properties are Cu, Zn, Au, Ti, and Ag [10]. NPs are demonstrated to have the most effective bactericidal properties against different pathogenic microbes, including bacteria, yeasts, fungi, and viruses [11].

The aim of this study was to evaluate the antimicrobial effects of edible nano-composite based on bean pod shell gum (as a novel source of polysaccharide gum), TiO₂ NPs and *Mentha pulegium* EO on five food-borne pathogens in two categories including two Gram-positives and three Gram-negatives bacteria.

2. MATERIALS AND METHODS

2.1. Gum Extraction Process

Bean pod was purchased from Neyshabour farms in Iran. After washing and peeling, the shell around the grains were separated and dried in a vacuum drier at 70°C and 133 mbar (Memmert VO400 model, Germany). Dried shells were grinded in an electrical mill, sieved by a sifter (mesh 250 micron), and kept at cool condition. Then, 100 g of bean pod shell powder was treated for three times with ethanol at a ratio of 1:10 in a hot water bath at 70°C for 2 hours to remove lipids, pigments, and saponins. The ethanol solution was filtered through Whatman 45 filter paper and the retentate was treated at 50°C by acetone at ratio of 1:10 for better purification. The remaining solid matters were washed with distilled water in a hot water bath at 70°C for 2 hours. The smooth solution was centrifuged (ABA model, Germany) for 15 minutes at 6,000 rpm for separation of insoluble components. The liquid part of centrifuge tube collected and concentrated by rotary evaporator (Heidolph Laborota 4003 Model, Germany) at 60°C, then treated by ethanol at the ratio 1:3 (concentrate:ethanol) to precipitate hydrocolloids which was then dried in vacuum oven [12].

2.2. Edible Film Preparation

For preparation of the edible film, we modified the method of Perez-Cordoba *et al.* [13]. Bean pod shell gum solution was prepared at 4% w/v concentration, followed by adding 85% glycerol (Merck-Germany), and 99% polyethylene glycol (Biomedical-Netherlands) in ratios of 2% and 4% v/v, respectively. The plasticizer glycerol was added and mixed into bean pod shell gum solution using a magnetic stirrer at 95°C for 15 minutes. Then, the *M. pulegium* EO and TiO₂ NPs (Sigma-Aldrich, Germany) were added at concentration of 2% and 4% v/v and 1% and 2% w/v, respectively. The plates including above treatments were taken in 37 incubators for 24–48 hours until drying the films. The treatments were repeated in triplicate and the sample without any NP and *M. pulegium* EO was considered as the control.

2.3. Preparation of Microbial Suspension

Five microbial strains, including Gram-positive and Gram-negative bacteria were prepared from American type culture collection (ATCC). *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 11776), *Escherichia coli* (ATCC 8739), *Salmonella*

typhoid (ATCC 14028), and *Pseudomonas aeruginosa* (ATCC 9027) were prepared in a plate count agar (Merck-Germany). Then using 0.5 Mc-Farland's solution, standard microbial solutions with a count of 1.5×10^8 CFU/ml were prepared in Muller Hinton Agar media [14].

2.4. Disc Diffusion Test

The method to determine inhibition zone was according to the Kirby *et al.* method with some modification. The edible coating prepared according to the 2.2 method was formed in 8-mm disks using punch and placed on lawn culture of plate count agar medium incorporated with 15 µl of each microbial suspension using sterile cotton swab. The plates were put at room temperature for about 1 hour to allow the solution to diffuse from the discs into the medium, and then incubated at 37°C for 24 hours and after that the diameter of the zone from microbial growth inhibition around each disk were measured and recorded in millimeter [15].

2.5. Statistical Analysis

The treatments were set using randomized design in triplicate (Table 1). Data of antimicrobial activity were analyzed using excel 2016 software and results of inhibition zones reported by $X \pm SD$. The differences between treatments were determined by analysis of variance and LSD tests at 95% using SPSS 2010 software.

3. RESULTS AND DISCUSSION

The antimicrobial effect of nano-composite based on treatments indicated in Table 1, was assayed on five food-born pathogenic microbes noted in 2.3. According to the data presented in Table 2 increasing *M. pulegium* EO and TiO₂ NPs content increased antimicrobial activity of the edible film based on bean pod shell gum, so that the treatment E (containing 4% v/v *M. pulegium* EO and 2% w/v TiO₂ NPs) led to the highest inhibition zone (11.8–15.2 mm) compared to treatment B (containing 2% v/v *M. pulegium* EO and 1% w/v TiO₂ NPs) with inhibition zone range of 9.8–11.5 mm.

Figure 1 shows the effect of treatments on clear zones of bacterial growth in bar chart form in which the statistical differences are indicated. Generally the inhibitory effect is in the order of E > C > D > B > A, and the difference is statistically significant except of some cases.

According to Figure 1, *S. aureus*, the Gram-positive bacterium, was more susceptible than Gram-negative bacteria against antimicrobial agent used. *B. cereus* was more resistant to the treatments, because of the spore-forming property. The higher

Table 1. List of treatments.

TiO ₂ NPs (% W/V)	<i>M. pulegium</i> EO (% V/V)	Bean pod shell gum solution (% W/V)	Treatments
0	0	4	A (control)
1	2	4	B
2	2	4	C
1	4	4	D
2	4	4	E

Table 2. Inhibition zones of different edible films based on bean pod shell gum (4% w/v), TiO₂ NPs (1%–2% w/v) and *M. pulegium* EO (2%–4% v/v) on food-borne pathogens.

Microorganism	Treatments				
	A* (control)	B	C	D	E
<i>S. aureus</i>	0	11.8 ± 0.6	14.2 ± 0.5	13.7 ± 0.6	15.2 ± 0.4
<i>B. cereus</i>	0	9.8 ± 0.2	10.9 ± 0.3	10.2 ± 0.5	11.5 ± 0.6
<i>E. coli</i>	0	6.5 ± 0.2	8.9 ± 0.4	7.6 ± 0.3	9.7 ± 0.7
<i>S. typhoid</i>	0	7.1 ± 0.5	8.9 ± 0.3	7.4 ± 0.4	9.6 ± 0.5
<i>P. aeruginosa</i>	0	7.5 ± 0.3	9.1 ± 0.4	8.8 ± 0.2	10.3 ± 0.5

*Treatments (A, B, C, D, and E) are presented in Table 1.

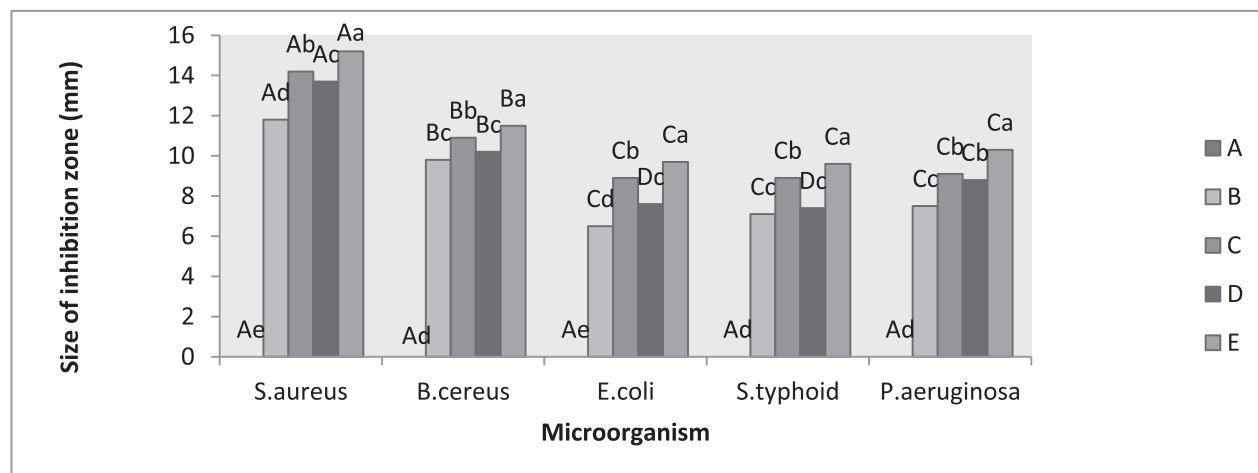


Figure 1: Comparison between inhibition zones of different edible films containing bean pod shell gum, TiO₂ NPs and *M. pulegium* EO on food-borne pathogens. *Means followed by the same lower case letters for each microorganism and capital letters between pathogens do not differ significantly by the LSD (Least Significant Difference) test ($p < 0.05$).

resistance of Gram-negative bacteria compared to Gram positives against external agents has been reported before which is due to the presence of lipopolysaccharides in cell membrane. This layer makes them inherently resistant to external agents, including antibiotics, detergent, and hydrophilic dyes. In contrast, presence of an outer peptidoglycan layer in Gram-positive bacterial cells which is an ineffective permeability barrier makes them more sensitive to the external agents [16]. The hydrophilic cell wall structure in Gram-negative bacteria is a barrier against hydrophobic components to penetrate to the microbial cell [17].

TiO₂ is an attractive photocatalyst because it is nontoxic, chemically stable, and generally Recognized as Safe (GRAS) and inexpensive [18]. The nano-sized titanium dioxide particles have higher photocatalytic activity than bulk form which is due to higher surface area [19]. Also, EOs have largely been used in different food systems for antimicrobial and antioxidant activities, but direct addition of the EOs may have adverse effects on sensory properties of the food. To overcome this side effect, EOs might be added into the edible films. The antimicrobial activity of EOs is related to their major phenolic compounds, such as thymol, eugenol, carvacrol, or terpenic compounds (α -pinene, β -pinene, 1,8-cineol, menthol, and linalool). Different types of EOs with different chemical compositions have different ability to bind the membrane proteins of microbial cells, and thus have different

inhibitory effects [20]. The antimicrobial efficacy of *Mentha* EO has been found to vary from moderate to significant often correlating with the composition of the oil. The main components of *M. pulegium* EO are pulegone (40%–50%) and menthone (20%–30%) [21].

Besides, the antimicrobial and biofilm formation preventive properties of *M. pulegium* EO against *Streptococcus* mutants and *Streptococcus pyogenes* *in vitro* and *in vivo* have also been assessed [22]. Our findings show good agreement with the mentioned studies and confirm improvement of antimicrobial activity of the biofilm, after enrichment with EO and NPs.

4. CONCLUSION

The findings of the current work indicate a potential use of bean pod shell gum film enriched with TiO₂ NPs and *M. pulegium* EO as an excellent types of biodegradable compounds to inhibit the growth of different food pathogens in *in vitro* system. Increasing *M. pulegium* EO and TiO₂ NPs content increased the antimicrobial activity of the edible film. As both of the additives are non-toxic and GRAS which improved the functional properties, including antimicrobial activity of the edible film, so the edible coating based on bean pod shell gum has the potential to be used as active packaging for fresh produce.

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