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Antimicrobial activities of chitosan-based edible films produced by adding different macrofungi extracts and plants essential oils

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Farklı makromantar ekstraktları ve bitki uçucu yağları eklenerek üretilen kitosan bazlı yenebilir filmlerin antimikrobiyal aktiviteleri

Abstract: This research aims to reveal the antimicrobial activity of chitosan-based edible films containing different mushroom extracts and plant essential oils. In this study, edible films were produced using the essential oils of *Satureja cuneifolia* Ten., *Mentha longifolia* (L.) Hudson subsp. *typhoides* (Brig.) Harley var. *typhoides* (L.) Hudson and extracts of *Amanita caesarea* (Scop.) Pers. and *Boletus reticulatus* Schaeff. collected from different localities of Osmaniye province. The antimicrobial activities of these films were investigated on *Escherichia coli* by using the Kirby-Bauer disk diffusion test. At the end of the research, it was determined that the edible film obtained by adding *S. cuneifolia* Ten. essential oil (3%) had the highest antimicrobial activity. And also, it could be said that all the edible films produced had antimicrobial activity.

Key words: Antibacterial activity, edible film, volatile, mushroom

Özet: Bu araştırma, farklı mantar ekstraktı ve bitki uçucu yağı içeren kitosan temelli yenilebilir filmlerin antimikrobiyal aktivitesini açığa çıkarmayı amaçlamaktadır. Bu çalışmada, Osmaniye'nin farklı lokalitelerinden toplanan *Satureja cuneifolia* Ten., *Mentha longifolia* (L.) Hudson subsp. *typhoides* (Brig.) Harley var. *typhoides* (L.) Hudson'un uçucu yağları ve *Amanita caesarea* (Scop.) Pers. ve *Boletus reticulatus* Schaeff. ekstraktları kullanılarak yenebilir filmler üretilmiştir. Üretilen bu filmlerin *Escherichia coli* üzerindeki antimikrobiyal aktiviteleri Kirby-Bauer disk difüzyon testi kullanılarak araştırılmıştır. Araştırma sonunda, *S. cuneifolia* Ten.'in uçucu yağının (%3) ilave edilmesiyle üretilen yenilebilir filmin en yüksek antimikrobiyal aktiviteye sahip olduğu belirlenmiştir. Bununla birlikte, üretilen tüm yenebilir filmlerin antimikrobiyal aktiviteye sahip olduğu söylenebilir.

Anahtar Kelimeler: Antibakteriyel aktivite, yenebilir film, aroma, mantar

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1. Introduction

The deterioration of synthetic plastic materials has been a major cause of the growing interest in edible films in recent years (Kumar et al., 2022). Plastics are among the waste elements that harm the environment when interacting with nature since they take a long time to break down in the natural world (Lakhiar et al., 2024). However, they swiftly deteriorate after being thrown away, edible coatings from consumable goods can improve food quality by extending its shelf life (Xu et al., 2005). Chitosan is one of the polymer materials that is commonly utilized in the creation of edible films (Ebrahimi et al., 2024; Singh et al., 2024). It is created by deacetylating chitin, which is found in large quantities in shrimp and crab shells (Priyadarshi et al., 2018; Campalani et al., 2024). Chitin becomes more reactive and soluble in diluted acetic and citric acids, as a result of the deacetylation process (Ma et al., 2021). A valuable and promising biomaterial, chitosan is a derivative of chitin and a helpful bioactive polymer that has gained attention recently (Safarzadeh et al., 2024). Important characteristics of the edible polymer chitosan include its adsorption, non-toxicity, biodegradability, and biocompatibility (Amin et al., 2024). Chitosan finds application in the food business because of its unique chemical and physical characteristics (Gutiérrez, 2017). Chitosan film is a viable substitute for commercial polymers used to prolong food shelf life because it is a rigid, flexible, semi-permeable substance that rips readily (Butler vd., 1996; Kittur vd., 1998). In recent years, studies on chitosan-based edible films have started to examine their antibacterial and antioxidant properties by adding essential oils derived from aromatic and medicinal plants (Altiok et al., 2010). The biological effects of essential oils differ since they are composed of a diverse range of chemicals (Wińska et al., 2019; Parveen et al., 2024).

Numerous experts have found that non-toxic biocompatible chitosan has antibacterial and antioxidant properties in earlier investigations (Begin and Calsteren, 1999; Xie et al., 2001; Rabea et al., 2003; Yen et al., 2008; Goy et al., 2009; Raafat and Sahl, 2009; Kong et al., 2010; Siripatrawan and

Harte, 2010; Leceta et al., 2013; Ke et al., 2021). Several investigations have been carried out to examine the antibacterial and antioxidant activities of mushroom extracts (Mau et al., 2002; Yang et al., 2002; Cheung et al., 2003; Gao et al., 2005; Gezer vd., 2006; Puttaraju et al., 2006; Barros et al., 2007; Jayakumar et al., 2009; Smolskaite et al., 2015). A few studies were conducted previously on edible films produced by adding essential oil and mushroom extract (Kumar et al., 2021; John, 2022; Kaya et al., 2022). However, there is no sufficient study in the literature on chitosan-based films containing the extracts of *Boletus reticulatus* Schaeff. and *Amanita caeserea* (Scop.) Pers. and the essential oils of *Satureja cunefolia* Ten., and *Mentha longifolia* (L.) Hudson *subsp. typhoides* (Brig.) Harley *var. typhoides* (L.) Hudson. This study aims to investigate the antimicrobial activities of chitosan-based edible films obtained by adding the extracts of *A. caesarea* (Scop.) Pers., and *B. reticulatus* Schaeff. macrofungi and the essential oils of *S. cunefolia* Ten., and *M. longifolia* (L.) Hudson *subsp. typhoides (Brig.) Harley var. typhoides* (L.) Hudson. plants collected from Osmaniye province.

2. Materials and Method

2.1. Sampling, extraction, production and characterization of edible films

The localities of plant and fungal species are given in Table 1. Edible film production steps and physical and chemical analyses of the produced films were mentioned in the studies conducted by Bülbül et al., (2023) and Gökyermez et al., (2023). Plant and fungal species were collected and identified by the last author.

2.2. Antimicrobial activity

In the study, *Escherichia coli* was used to determine the antimicrobial activities of the edible films. The Kirby-Baurer disc diffusion assay was used to determine the antibacterial activities of the produced edible films. According to the assay, the commercial Müller-Hinton Agar (MHA, Merck) medium was autoclaved at 121°C. After the processing, the obtained hot liquid (70 °C) medium was poured into sterile petri plates of 9 cm diameter. After the cooling of medium, The bacterial strain was spread on the surface of the rigid MHA plate with a swab and pre-incubated for 10 minutes. The inoculated samples were incubated at 25 °C for 15 minutes and then at 37ºC for 24 hours. The resulting inhibition zones were measured with a ruler in mm (Sedefoğlu et al., 2023). Assays were performed in three replicates.

2.3. Statistical analysis

The results were evaluated using the one-way analysis of variance Duncan test (95% confidence interval) with the help of the Statistical Package for the Social Sciences (SPSS) program (IBM Statistics, USA) (Version 18.0). In addition, the effects of the variables on the outputs were determined by two-way analysis of variance.

3. Results and Discussion

The research by Bülbül et al. (2023) and Gökyermez et al. (2023) displayed the edible films' physical (thickness, moisture content, water solubility, opacity, UV-Vis, FTIR, and SEM) and chemical analysis (total phenolic compound content, DPPH activity) results. However, *E. coli* was used to examine the films' antibacterial properties in the present study. As seen in Figure 1, it was determined that antimicrobial activity increased with the increase in the amount of mushroom extracts and essential oils added to the edible films. It was determined that the edible film obtained by adding *S. cuneifolia* Ten. essential oil (3%) had the highest antimicrobial activity. And also, it could be said that all the edible films produced had antimicrobial activity.

In several previous studies, chitosan-based edible films were produced using different samples, and their various activities (antioxidant and antimicrobial activities, etc.) were investigated (Yuan et al., 2016; Hromiš et al., 2017; Kaya et al., 2018; Xu et al., 2021; Sarfraz et al., 2024). It was determined that these produced films had antioxidant and antimicrobial activities.

Gómez-Estaca et al. (2009) produced a chitosan-based film utilizing clove oil and used this film to preserve raw sliced salmon and investigated its antibacterial properties against the bacterial strains (*L. acidophilus, P. fluorescens, L. innocua* and *E. coli*.) At the end of the study, a decrease in total bacterial growth was observed after 11 days of storage, and therefore it was stated that, the produced films are suitable for use as an active packaging substance applied to fish products. In the study conducted by Sánchez-González et al. (2009), chitosan-based edible films including different concentrations of *Melaleuca alternifolia* (Maiden & Betche) Cheel essential oil, were obtained and the water vapor permeability, mechanical, and optical features of dried films were investigated to evaluate the effect of incorporating essential oil into these films. In addition, composite films were photographed by utilizing a scanning electron microscope (SEM) and their antibacterial features were tested against two bacterial strains (*Listeria monocytogenes* and *Penicillium italicum*). It was found that, chitosan-based films have a significant antimicrobial activity. Handayasari et al (2019) produced chitosan-based films containing garlic oil and nitrite-added gelatin-chitosan. They also investigated the mechanical properties and antibacterial effect of the films. At the end of the study, the tensile strength of the film increased gradually with the increasing amount of chitosan, but the elongation at break decreased. Furthermore, with the addition of the oil, the antibacterial activity of the films increased significantly. In

Table 1. Localities of plant and fungal species

Samples	Localities	GPS coordinates	Collection Date
Mentha longifolia subsp. *typhoides* var. *typhoides*	Bahçe, Yukarı Kardere village, Mandal deresi place	37º 13'K, 36º 37'D, 878 m,	10.07.2021
Satureja cuneifolia	Düziçi, Baskonuş high plateau, Odunluk place	37º 21'K, 36º30'D, 1681 m,	07.09.2021
Amanita caesarea	Osmaniye, Zorkun high plateau, Karıncalı place	36º57'K, 36º20'D, 1324 m	08.10.2021
Boletus reticulatus	Osmaniye, Zorkun high plateau, Karıncalı place	36º57'K, 36º19'D, 1240 m	08.10.2021

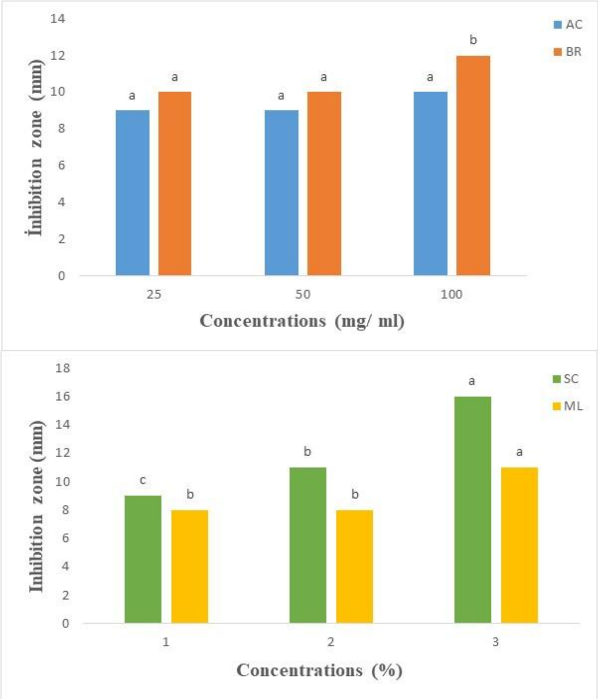

Figure 1. Antimicrobial activities of chitosan-based edible films (AC: *Amanita caesarea*, BR: *Boletus reticulatus*, SC: *Satureja cunefolia*, ML: *Mentha longifolia* subsp. *typhoides* var. *typhoides*). Mean with different letter in columns is significant at p<0.05.

the study by Şimşek et al. (2020), essential oils of *Eucalyptus globulus* Labill., *Schinus molle* L., and *Santolina chamaecyparissus* L. gathered in Osmaniye (province of Türkiye) were extracted by using a Clevenger apparatus. Carboxy-methyl cellulose films were obtained by utilizing different concentrations of the oils to investigate the physical, chemical, and antibacterial effects of these films. The conclusion of the study revealed that films with added essential oil exhibited significant changes in their physicochemical properties and antimicrobial effects compared to the control groups. In another study, the chitosan-based films with the included water-based extract of *Tricholoma terreum* (Schaeff.) P. Kumm. gathered from the İnönü plateau in Sakarya province of Türkiye, were produced by Koç et al (2020). The phenolic content, optical transparency, thermal analysis, thermo-gravimetric analysis, functional groups by FTIR, water contact angles, and solubility in water, along with antioxidant, antimicrobial, and anti-quorum sensing activities were tested to determine the potential of the films. As a result of this study, the phenolic content of 0.1 g of fungi extract included 2659.82 μg g^{-1} of p-coumaric acid and 2126.69 μg g^{-1} of gallic acid. Moreover, the researchers found that, the chitosan-based films including fungi extract have a thickness of 190 μm and a light brown color. Furthermore, the films have optical transparency ranging from 17% to 53% and water contact angles of 76.06 degrees. Lastly, the elongation of break lengths of the films was determined to increase by 111.1%, when compared to the control group. In terms of antioxidant activity, the films had more than double the effect of the control group. Similarly, the study showed that the films had remarkable antimicrobial activity compared to the control group as well. In the research conducted by Savin et al. (2020), chitosan-based films with added extract of *Ganoderma lucidum* (Curtis) P. Karst., which was obtained from Romania medica SRL laboratories exhibited substantial effects of antioxidant, cytotoxic, and antimicrobial. It was revealed that, the films had more effective antimicrobial activity against Gram-positive bacterial strains than against Gram-negative bacterial strains and that in terms of antioxidant tests such as ABTS and DPPH, the films showed a moderate level of activity.

Conflict of Interest

The authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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