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Extraction and Characterization of Bioactive Compounds from *Prunus Spinosa L.* Fruits with Potential in the Development of Edible Films

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RESEARCH ARTICLE

Abstract

Prunus spinosa L., commonly known as blackthorn, is rich in bioactive compounds such as flavonoids, anthocyanins, phenolic acids, vitamins, minerals, and organic acids, displaying strong antioxidant and antibacterial properties. This investigation aims to assess bioactive compounds in fresh and lyophilized blackthorn fruits and analyze ethanolic extracts for phenolic content, antioxidant activity, and antimicrobial effects. The primary objective was to develop a chitosan-based film with antimicrobial and antioxidant properties, targeting the enhancement of food packaging solutions, along with inhibition of *S. aureus* bacteria. Results showed that ethanolic extracts of blackthorn possess significant antimicrobial efficacy, demonstrating notable effectiveness against *S. aureus*. Notably, the film incorporating an 80% ethanolic extract demonstrated greater effectiveness against *S. aureus*-inoculated cheese. This outcome underscores the inhibitory potential of blackthorn extracts on *S. aureus* growth within a food matrix.

Keywords: antioxidant, antibacterial properties, blackthorn, *Prunus spinosa* L., chitosan

INTRODUCTION

Cultivated extensively in regions such as New Zealand, Tasmania, and the eastern part of North America, *Prunus spinosa L.*, commonly referred to as blackthorn, is a thorny tree that reaches moderate heights (Popescu, I., Caudullo, G., 2016). Blackthorn fruits contain a high amount of bioactive components such as flavonoids, anthocyanins, phenolic acids, vitamins, minerals, and organic acids; these compounds possess notable antioxidant and antibacterial characteristics. Notably, flavonoids like catechin, epicatechin, and rutin have been linked to diabetes protection, whereas other flavonoids, including myricetin, quercetin, and kaempferol, have been linked to antihypertensive activity. Due to their simplicity, efficiency, and widespread applicability, solvent extraction methods are frequently employed for the extraction of phenolic chemicals from plant sources (Natic. et al., 2019; Ürkek et al., 2019, Luna-Vázquez et al., 2017, Lall et al., 2015; Verma et al., 2013; Santos et al., 2013). Consumer interest in healthier foods free

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of artificially generated additives is a relatively new phenomenon. This trend has sparked curiosity regarding incorporating naturally occurring substances exhibiting antimicrobial and antioxidant properties. Interestingly, this inclination towards natural remedies has historical roots; centuries ago, blackthorn was employed for medicinal purposes in disease treatment and prevention (Kultur, 2007).

Previous research into the biological properties of *P. spinosa L.* fruits has primarily focused on their antioxidative capacities [Lapidot et al., 1999; Fraternale et al., 2009; Ruiz-Rodriguez et al., 2014, Opriș et al., 2021; Marcetic et al., 2022; Veličković et al., 2021, Sikora et al., 2013]. Furthermore, there is a limited body of knowledge about their antidiabetic [Temiz and Temur, 2019; Stankovic et al., 2022; Crnić et al., 2021], antibacterial [Gündüz, 2013; Smullen et al., 2007; Veličković I. et al., 2021; Velickovic J. et al., 2014], anti-inflammatory [Cosmulescu et al., 2017; Fraternale et al., 2009; Ganhao et al., 2010; Radovanović et al., 2013; Varga et al., 2017; Veličković et al., 2016; Veličković et al., 2016;], and anticancer [Olszewska et al., 2001; Kello et al., 2017; Condello et al. 2019] activities. The findings highlight the significant value of blackthorn fruits as healthy consumables with health-promoting properties. According to Fraternal et al. (2009), these fruits can be beneficial to the food industry, particularly the creative sector, in terms of dietary supplements and the development of novel ingredients with enhanced antioxidant capacities.

Research has demonstrated that integrating extracts and natural constituents sourced from plants, spices, and herbs into packaging and coating films results in enhancements of active properties such as antioxidants, antimicrobials, and anti-browning effects (Gomez-Guillen et al., 2007; Siripatrawan et al., 2010; Wang et al., 2012). Extracts enriched with valuable chemical compounds that exhibit antioxidant and antimicrobial characteristics can confer added value to food items by extending the shelf life of products enclosed with these films. This enrichment extends the product shelf life using these films (Li et al., 2012). The presence of antioxidants plays a crucial role in averting the oxidative degradation of lipids, essential oils, fat-soluble vitamins (A, D, E, and K), as well as pigments (Valenzuela et al., 2003). Among these compounds, flavonoids stand out, showcasing potent antioxidant properties that yield beneficial impacts on human well-being, as Pereira et al. (2012) noted. Phenolic acids, such as gallic acid, are another noteworthy category, manifesting both antioxidant and anti-browning capabilities. This dual functionality has been linked to their adeptness in reducing ortho-quinones into colorless phenolic compounds (Kubo et al., 2003).

This investigation aimed to assess bioactive compounds in fresh and lyophilized blackthorn fruits and analyze ethanolic extracts for phenolic content, antioxidant activity, and antimicrobial effects. The primary objective was to develop a chitosan-based film with antimicrobial and antioxidant properties, targeting enhancing food packaging solutions.

MATERIALS AND METHODS

Reagents

During the experiments, all reagents utilized were of analytical grade. These included ethanol, methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), and Folin-Ciocâlteu agent, all procured from Sigma-Aldrich (Steinheim, Germany). For the formulation of films, the materials employed included high molecular weight (98–99%) hydrolyzed PVA (polyvinyl alcohol), chitosan, and itaconic acid, which were sourced from ThermoFisher based in Kandel, Germany. Additionally, glycerol was acquired from VWR Chemicals (International Ltd).

Plant material

To conduct the analyses, mature blackthorn fruits were gathered from Bogdana village, Sălaj county (47°01'34.5" N, 23°00'34.7" E). These harvested samples were subsequently stored in a freezer at a temperature of -18°C until they were analyzed.

Freeze-Drying Process

Freeze-drying was carried out using a Telstar LyoQuest -55 Plus (Terrassa, Barcelona) apparatus with a condenser at -55°C. Blackthorn fruits were placed in a 1000 mL flask (1:3 ratio) and frozen at -80°C for 24 hours. The lyophilization process took place for 72 hours under vacuum conditions of 0.001 mBar at a temperature of -55°C. Samples were dried until a constant weight (±0.005 g) was achieved.

Extracts preparation

To prepare ethanolic/methanolic extracts, 1 g of the sample was weighed and combined with 10 ml of EtOH/MeOH in centrifuge tubes. The mixture underwent 30 minutes of ultrasonication for enhanced extraction. Following ultrasonication, centrifugation at 6000 rpm for 10 minutes using a Sigma model 2-5 centrifuge (Osterode, Germany) was performed. The resulting supernatant was collected and subjected to repeated extractions until discoloration occurred. The supernatant was then filtered through 0.45 µm Millipore filters. Using a Rotavap Laborata 4010 Digital from Heidolph (Schwabach, Germany), the supernatant was evaporated to dryness, and the resulting extract was reclaimed in 3 ml of methanol, subsequently filtered through 0.45 µm Millipore filters. The obtained extracts were stored at -18°C, intended to analyze biologically active compound content in subsequent practical endeavors.

Total Polyphenol Content

The analyzed extracts' total phenolic content (TPC) was determined using the Folin-Ciocâlteu method. 100 µl filtered methanolic extract was mixed with 6 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent. After 5 minutes, 1.5 ml of Na_2CO_3 solution (7.5%) was added to create a base environment (pH ~10) required for the reaction between phenolic compounds and the Folin-Ciocâlteu reagent. After 90 minutes of incubation at room temperature, absorbance was measured at 750 nm using a UV-VIS spectrophotometer (Shimadzu UV-1700 PharmaSpec; Kyoto, Japan). A reference sample was prepared using the same procedure, replacing the extract with methanol. The samples were analyzed in triplicate.

Antioxidant Activity of Extracts before and after Lyophilization

The samples' antioxidant activity was assessed both before and after the lyophilization process using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Fresh DPPH solution was prepared in 95% methanol. From this solution, 3.9 ml was taken and combined with 10 µl of methanolic sample extract and 90 µl of water. After homogenization and a 30-minute dark incubation, absorbance was measured at 515 nm. For calibration, the device was initially set with methanol at 515 nm to achieve an absorbance of 0. Subsequently, the sample reading was taken with the help of a UV-VIS spectrophotometer (Shimadzu UV-1700 PharmaSpec; Kyoto, Japan). The samples were analyzed in triplicate.

Film Preparation

The films were fabricated through the casting method, employing the procedural approach detailed by Teleky et al. (2022) with minor modifications. The film-forming solution was created by blending 2 g of biopolymer and glycerol (30% w/w biopolymer) in 100 mL of distilled water at 90°C. Glycerol was dissolved using an Ultraturax Polytron PT 6100 D, Kinematica (Malters, Switzerland) at 9500 rpm for 30 seconds. The biopolymer was gradually added and homogenized for 5 minutes at 9500 rpm, then 5 minutes at 15000 rpm. Two flasks were used to hold separate solutions of ethanol extracts, one with a concentration of 50% ethanol and the other with a concentration of 80% ethanol. The mixture was cooled overnight for complete biopolymer hydration. The solution was treated at 55 ± 5 °C for 30 minutes in an ultrasonic water bath to eliminate air bubbles. The next step involved adding 15 g of the film-forming solution to Petri dishes. The samples were left at room temperature for 4 days to evaporate the solvent. The resulting films were stored in opaque zip lock bags in darkness until further analysis. After this procedure, 3 types of films were obtained: control film (with no extract), films with an extract of 50% ethanol, and films with an extract of 80% ethanol.

Film Characterization- Physical Measurements

Film thickness (mm) was evaluated using a digital caliper (Lumytools LT15240, Suceava, Romania) at five random spots for each film. This measurement process was carried out in duplicate. The overall thickness was determined as the mean value of all the measurements. Sample diameter (mm) was assessed using the same digital caliper at three random points. This step was performed in duplicate. For film mass (g), an analytical balance (Sartorius-Acculab Atilon, Göttingen, Germany) with a precision of 0.0001 g was employed. The ultimate weight was established as the average of two measurements. The measurements were done in accordance with Teleky et al. (2022).

Antimicrobial Activity of Extracts and Film Solutions - MIC, MBC

The antimicrobial effectiveness of the ethanolic extracts was assessed against several pathogens, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Salmonella enteridis* ATCC 13076. The goal was to determine the minimum inhibitory concentration (MIC) following the National Committee for Clinical Laboratory Standards guidelines, as detailed in Cătunescu et al. (2019). The minimum inhibitory concentration (MIC) was determined using a 96-well microtiter plate. In the first well, 100 µl of sterile nutrient broth and 100 µl of the sample were combined, along with 10 µl of bacterial inoculum (1.5×10^4 CFU ml^{-1}). Gentamicin (0.4 mg ml^{-1} in saline) was a positive control, and the extraction solvent acted as the negative control (Cătunescu et al., 2019). After 22 hours of incubation at 37°C, 20 µL of 0.2 mg/mL resazurin aqueous solution was added to each well. The MIC, representing the concentration that halted bacterial growth, was identified by the well where the blue color remained unaffected.

Preparation of bacterial strains

Following the protocol by Teleky et al. (2022), bacterial strains were prepared by transferring a small number of colonies from each strain's growth medium to a sterile NaCl solution (9 ml). The suspension was adjusted to attain a turbidity equivalent to McFarland standard 0.5 (1.5×10^8 CFU/mL). Subsequently, a bacterial suspension with a concentration of 1.5×10^4 CFU/mL was added to each microplate.

The MIC of the film solution was determined using an antibacterial assay with resazurin microtiter plates. Each well of a 96-well microplate received 100 µl of specific sterile growth broth medium. The initial wells contained 100 µl of each extract and the prepared films, followed by 11-fold serial dilutions in subsequent wells. After adding 10 µl of inoculum (1.5×10^5 CFU/mL) to all wells, gentamicin (0.4 mg/ml in saline) served as the positive control, and film solution without extract acted as the negative control. Microplates were incubated for 20-22 hours at 37°C. Subsequently, 20 µL of a 0.2 mg/mL aqueous resazurin solution was added to each well, followed by a further 2-hour incubation at 37°C. During this period, viable bacterial cells transformed resazurin (non-fluorescent blue dye) into resorufin (fluorescent pink) in wells containing them, as per Teleky et al. (2022).

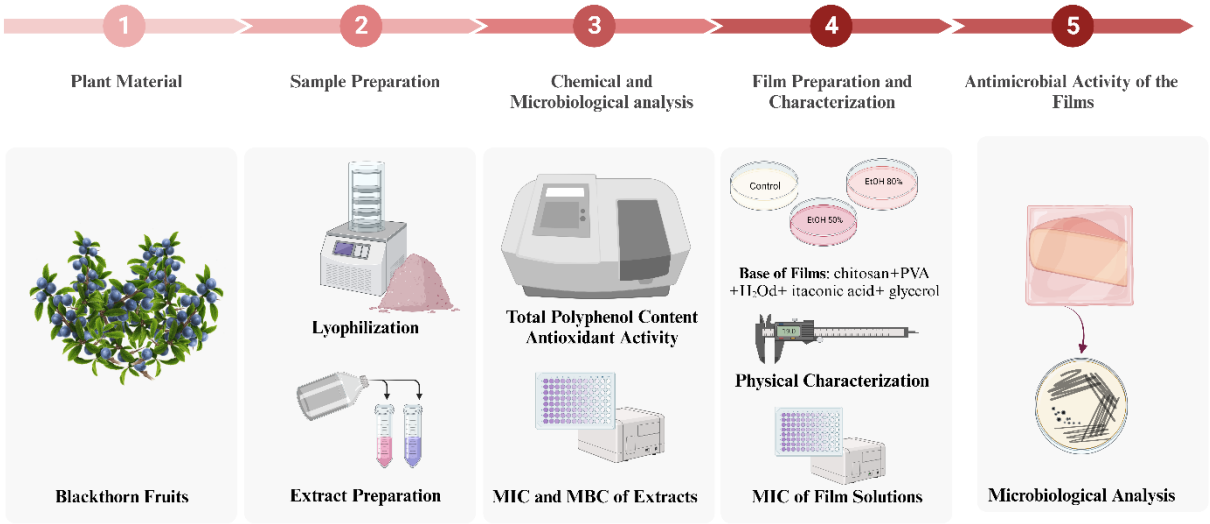

Figure 1. Main steps towards obtaining the films with blackthorn extracts. Created with BioRender.com

Film Antimicrobial Efficiency on Food Matrix- Cheese

Sliced cheese (approximately 9,5x9,5x2 cm) was purchased from a local grocery store (Cluj-Napoca, România). Initially, cheese slices were cut to dimensions of 85x85x2 mm and arranged in Petri dishes. Cultures were prepared in advance for inoculation onto the cheese. Among the 28 plates containing cheese, 26 received inoculation with 1 ml of culture: 13 with *E. coli* and 13 with *S. aureus* culture. Two plates were reserved for the negative control. Within the 13 samples specific to each culture, the following were added over the film after inoculation: 4 with the control film, 4 with the film containing 50% ethanol extract, and 4 with the film containing 80% ethanol extract. Additionally, one plate without a film, solely with the inoculum, represented the positive control. After this step, the Petri dishes were subjected to incubation (37 °C, 24 h). The final bacterial cell concentration in the 1 ml inoculum used for those 26 samples was 10^6. (Park et al., 2020).

The examination of the efficiency of the films on cheese was realized according to Park et al., 2020 and adapted to our application. After incubation, 3 g of each of the 28 cheese samples were aseptically collected and homogenized with 45 mL of physiological saline solution. Subsequently, the homogenized samples' test tubes were subjected to vortexing to ensure thorough mixing. From each sample, 100 µl of the resulting solution was aseptically transferred and evenly spread on selective culture media appropriate for the target microorganisms, such as Baird-Parker agar and TBX medium. The inoculated plates were then incubated under controlled conditions at 37 °C for 24 hours. Following incubation, the plates were carefully examined for the presence of microbial growth and any characteristic colonies or indicators of microbial activity. The process was repeated after 4 days until there were no significant differences.

RESULTS AND DISCUSSIONS

Total Phenolic Content and Antioxidant Activity

Polyphenols are molecular structures constituted by hydroxyl groups directly linked to a central aromatic core. We initially extracted these compounds using ethanol and methanol solvents to evaluate the levels of polyphenols and the antioxidative prowess within frozen blackthorn and blackthorn powder samples. From the five blackthorn samples (both fresh and freeze-dried) subjected to the Folin-Ciocâlteu method for assessing the total polyphenol content, the highest value was observed in the frozen blackthorn sample treated with an ethanolic extract (2047.16

mg GAE/100 g dw). As for the samples treated as lyophilized powders in 50% ethanol, 80% ethanol, and methanol, the most substantial quantification was seen in the 80% ethanolic extract (1962.64 mg GAE/100 g dw).

The samples were analyzed in duplicate and quantified using a calibration curve established with varying concentrations of gallic acid solutions. The total polyphenol content of both fresh and lyophilized blackthorn samples was expressed as milligrams of gallic acid equivalents per 100 grams of the sample, considering the dry weight.

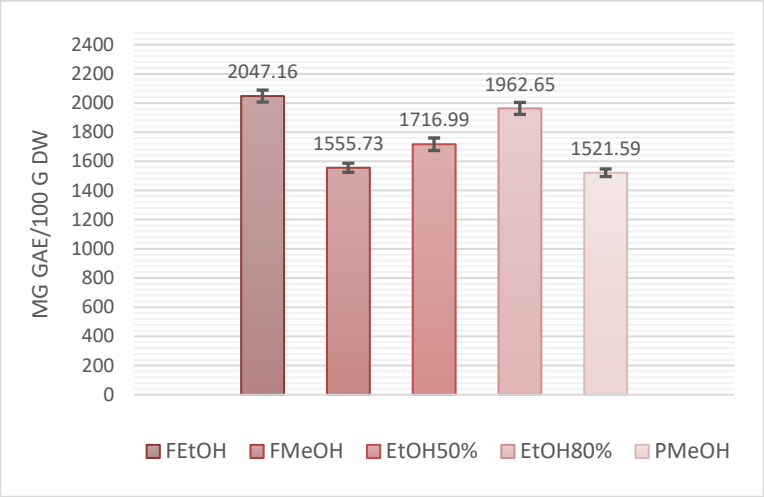

Figure 2. Total Polyphenol Content of Fresh and Lyophilized Blackthorn

Legend**:** FEtOH-Ethanolic Extract of Fresh Blackthorn; FMeOH-Methanolic Extract of Fresh Blackthorn; EtOH50%-50% Ethanol Extract of Lyophilized Blackthorn; EtOH80%-80% Ethanol Extract of Lyophilized Blackthorn; PMeOH-Methanolic Extract of Lyophilized Blackthorn

According to previous investigations, the content of TPC in ethanol extracts of blackthorn fruits from Romania was 1.02 mg GAE/g DW for 40% ethanol. The researchers observed that the total content of the extracted polyphenols decreased with increasing ethanol concentration (Opriş et al., 2021). Nevertheless, in our study, the TPC increased with the increment of ethanol concentration (1716,99 mg GAE/ 100 g dw to 1962,64 mg GAE/100 g dw). Contradictory findings regarding ethanol's impact on blackthorn polyphenol extraction highlight potential methodological, sample, and chemical complexity influences.

The antioxidant activity of the samples analyzed is presented as percentage inhibition capacity (%RSA), and it is shown in Figure 3. The findings from evaluating the antioxidant activity of the lyophilized blackthorn powder indicate that the 80% ethanolic extract demonstrates the highest antioxidant potency (73.07 %RSA) among all three samples (lyophilized powder in 50% ethanol, in 80% ethanol and methanol). These outcomes correlate with the results obtained for total polyphenol content, where the 80% ethanolic extract displayed a substantial amount of total polyphenols.

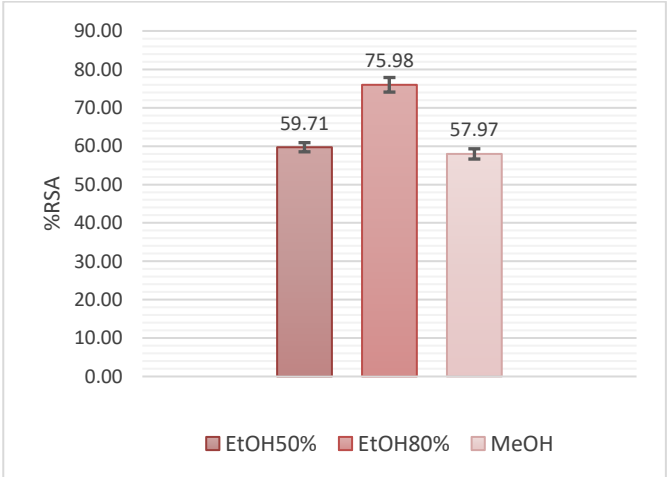

Figure 3 Antioxidant Activity of Lyophilized Blackthorn

Legend: EtOH50%-50% Ethanol Extract of Lyophilized Blackthorn; EtOH80%-80% Ethanol Extract of Lyophilized Blackthorn; MeOH-Methanolic Extract of Lyophilized Blackthorn

Opriş et al. (2018) mentioned in their study that the best antioxidant activity (52.57±5.87 mM Trolox/g DW) was found for 30% ethanol extract with an extraction time of 45 minutes. To our knowledge, there are currently no other studies on blackthorn powder antioxidant activity besides Opriş et al. (2018), but there are studies on fruits from the same family (*Prunus*). One of the study samples utilized by Coman et al. (2018) consisted of plum peel powder, upon which several tests, including an assessment of antioxidant activity, were conducted. The 70% ethanolic extract of plums showed 35.01 ± 4.09 % inhibition of DPPH. Freeze-dried chokecherry's antioxidant capacity was 49.188 ± 1.57 % of DPPH discoloration. Another study investigated the antioxidant activity of chokecherries (*Prunus virginiana* L.), revealing that freeze-dried fruits exhibited an antioxidant capacity of 49.18 ± 1.57% DPPH inhibition (Téllez-Pérez et al., 2020).

Microbiological Activity of Blackthorn Extracts

To assess the antimicrobial activity, the vulnerability of the ethanolic extracts was examined against three bacterial strains: two gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 25922) and one gram-positive strain (*Staphylococcus aureus* ATCC 6538P). Based on the obtained outcomes, **Table 1** presents that the gram-positive bacterium *S. aureus* is responsive to the antimicrobial agents found in extracts 1 (ethanol 50%) and 2 (ethanol 80%), both in terms of their bacteriostatic and bactericidal effects. Moreover, the gram-negative bacterium *E. coli* is susceptible to the antimicrobial agent present in extract 2, with regards to its bacteriostatic impact. At the same time, *Salmonella enteritidis* displays an intermediate susceptibility to the two extracts. Its antimicrobial mechanism involves the disruption of bacterial cell membranes or the inhibition of essential cellular processes, impeding bacterial growth (Kumar et al., 2021;Yu et al., 2022).

Table 1 MIC and MBC Results of Blackthorn Extracts

Sample	*Staphylococcus aureus* ATCC 6538P		*Salmonella enteritidis* ATCC 13076		*Escherichia coli* ATCC 25922	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
EtOH50%	2,4	2,4	11	11	5	-
EtOH80%	2,4	2,4	22	22	2,4	-
Gentamicin (mg/ml)	0,00005		0,00024		0,0005	

Note: EtOH50%- 50% ethanolic extract from the lyophilized powder; EtOH80%- 80% ethanolic extract from the lyophilized powder

Microbiological Activity of Film Solutions

Before testing the films in the food matrix, we conducted a microbiological assessment on the film-forming solutions to evaluate their impact on *E. coli* and *S. aureus* bacteria.. The findings presented in **Table 2** indicate that both the film-forming solution containing 50% ethanol extract and the one with 80% ethanol extract exhibit antimicrobial properties against *S. aureus*. Based on these outcomes, the decision was made to proceed with microbiological analyses specifically targeting the efficacy of the films solely on food matrices that were inoculated with *S. aureus*.

Table 2 MIC Results of Film Solutions

Sample	*E. coli*	*S. aureus*
FS-EtOH50%	-	0,5 %
FS-EtOH80%	-	0,2 %
Gentamicin(0,5mg/ml)	0,098 µg/ml	0,195 µg/ml
Chitosan Solution	-	-

Note:FS-EtOH50%- Film Solution with 50% ethanolic extract from the lyophilized powder; FS-EtOH80%- Film Solution with 80% ethanolic extract from the lyophilized powder

Physical Characterization

Before conducting physical measurements utilizing a caliper and an analytical balance, the films underwent a visual examination, revealing a compact structure devoid of internal bubbles.

As shown in Table 3, the physical characterization results reveal several important details about the films created using different samples. The film diameters range from 8.35 to 8.53 cm. These measurements indicate that the

size of the films is relatively consistent across the different samples, with only a slight variation. The weight of the films falls within the range of 0.93 to 1.29 grams. There is some variability in weight among the samples, with the control film being the lightest and the F-EtOH80% film being the heaviest.

The thickness of the films ranges from 0.15 to 0.19 mm. Similar to the diameter, the thickness also shows relatively minor variations among the samples.

Overall, these results suggest that the different samples used in creating the films have a limited impact on the resulting films' size, weight, and thickness. The variations observed are relatively small and may not be of significant practical consequence for the intended applications of these films.

Table 3. Physical Characterization of the Films

Sample	Diameter (cm)	Weight (g)	Thickness (mm)
Control	8.35633 ±0.51	0.939 ±0.05	0.15917± 0.01
F-EtOH50%	8.47283 ±0.85	1.24908 ±0.01	0.1975 ± 0.02
F-EtOH80%	8.537 ± 0.74	1.29325±0.02	0.1958 ±0.02

Note: F-EtOH50%- Film with 50% ethanolic extract from the lyophilized powder; F-EtOH80%- Film with 80% ethanolic extract from the lyophilized powder

Antimicrobial Activity of the Films

Compared to the positive control, which solely exhibits cheese inoculated with *S. aureus*, the cheese slices covered with the film showcase a reduction in colony-forming units. According to the results presented in **Tabel 4**, it's noteworthy that from T_0 (day 0 of testing) to T_7 (day 28 of testing), the films significantly curtailed the growth of *S. aureus* on the cheese, particularly the film-coated cheese containing the 80% ethanol extract, which saw a decrease from 4.61 $\log_{10}$ CFU/g to 1.38 $\log_{10}$ CFU/g. However, it's important to mention that the assessment of antimicrobial activity against *E. coli* is not provided due to the films not displaying significant inhibition against Gram-negative bacteria. The reasons for this could be related to differences in the susceptibility of *E. coli* compared to *S. aureus* to the antimicrobial components present in the films, or it might be influenced by the specific conditions of the test environment (Xavier et al., 2022; Tyasningsih et al., 2022).

According to Regulation (EC) No. 2073/2005 issued by the Commission on November 15, 2005, which outlines microbiological criteria for food products, the presence of *S. aureus* in cheese is permitted within the range of 100 to 1000 CFU/g (Regulation (EC) No. 2073/2005).

Table 4 Results of the Examination of the Efficiency of Films on Cheese Inoculated with *S. aureus*

Sample/ Day of testing	**T0** ($\log_{10}$ CFU/g)	**T1** ($\log_{10}$ CFU/g)	**T2** ($\log_{10}$ CFU/g)	**T3** ($\log_{10}$ CFU/g)	**T4** ($\log_{10}$ CFU/g)	**T5** ($\log_{10}$ CFU/g)	**T6** ($\log_{10}$ CFU/g)	**T7** ($\log_{10}$ CFU/g)
Control –	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Control +	4.64	4.47	4.42	4.38	4.35	4.33	4.32	4.30
F-Control	4.69	4.32	4.10	3.94	3.62	3.60	3.57	3.56
F-EtOH50%	4.72	4.35	3.51	2.86	2.27	2.10	1.63	1.44
F-EtOH80%	4.61	4.20	3.06	2.38	1.96	1.62	1.41	1.38

Note: Control- - Cheese without inoculum and without film; Control+ - Cheese with inoculum; F-Control- Control film; F-EtOH50%- Film with 50% ethanolic extract from the lyophilized powder; F-EtOH80%- Film with 80% ethanolic extract from the lyophilized powder: T0- day 0; T1- day 4; T2- day 8; T3- day 12; T4- day 16; T5-day 20; T6-day 24; T7- day 28; n.d- not detected

CONCLUSIONS

In this paper, UV-VIS spectrophotometric analyses facilitated the identification and quantification of antioxidant compounds (polyphenols) within ethanolic and methanolic extracts of blackthorn. The findings support that blackthorn fruits can be considered an important source of bioactive chemicals with significant antioxidant capabilities.

Using food-grade blackthorn ethanolic extracts and chitosan to create edible films proved favorable attributes that align well with potential applications in the food industry.

Microbiological investigations unveiled substantial antimicrobial potential within the ethanolic blackthorn extracts, particularly those at 50% and 80% concentrations, showcasing impressive activity against *S. aureus*, a frequently encountered pathogenic microorganism in food. However, these extracts demonstrated limited effectiveness against *E. coli*.

To evaluate the efficacy of chitosan-based films enriched with blackthorn extracts within a food matrix, we selected cheese as the test medium. After applying cheese inoculated with *S. aureus*, films formulated from 50% and 80% ethanolic extracts displayed a notable reduction in colony-forming units. Remarkably, the film containing 80% ethanolic extract demonstrated greater effectiveness. This outcome underscores the inhibitory potential of blackthorn extracts on *S. aureus* growth within a food matrix.

Author contribution: A.F., O.P., and O.N. conceived and designed the experiments; O.N., C.C., M.M., and E.S. performed the investigations, collected the data and interpreted the results; O.N. wrote the original manuscript; A.F., O.P., and S.S. supervised the experimental work and revised the original draft of the manuscript.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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