Design of an antibacterial medical face mask with oleuropein additive

DOI: 10.35530/IT.077.02.202528

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ABSTRACT – REZUMAT

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This study seeks to develop a protective mask infused with oleuropein to ensure excellent defence against bacteria and viruses. The mask features a three-layer construction, with the inner and outer layers composed of PP spunbond nonwoven fabric, while the centre layer consists of PP (polypropylene) meltblown nonwoven fabric. The leaves of olive trees in the Antalya region are harvested, air-dried, and subsequently pulverised into fine particles. Ground olive particles are removed utilising a Soxhlet apparatus. The extracted material is treated with a mask cloth using a method called "soak-hold-dry", and then it is tested for antibacterial activity according to the AATCC 147 standard. Staphylococcus aureus bacteria are selected to represent the gram-positive group, while Escherichia coli bacteria represent the gram-negative group. The zone diameters are measured at 47 mm for S. aureus and 40 mm for E. coli. The findings indicated that fabrics infused with olive leaf extract, which contains oleuropein, exhibit significant antibacterial efficacy. The average bacterial filtering effectiveness of masks without an oleuropein additive is 96.7%, but masks with an oleuropein ingredient have an efficiency of 98.5%. The mean breathability rose from 3.7 mm H_2O *to 4.8 mm* H_2O*. This data indicates that the breathability performance diminished with the addition of oleuropein.*

Keywords: *olive leaf, antibacterial activity, face mask, oleuropein*

Proiectarea unei măști medicale antibacteriene cu adaos de oleuropeină

Acest studiu urmărește să dezvolte o mască de protecție impregnată cu oleuropeină, pentru a asigura o apărare excelentă împotriva bacteriilor și virusurilor. Masca are o structură cu trei straturi, straturile interioare și exterioare fiind compuse din nețesut PP consolidat la filare, iar stratul central din material nețesut PP (polipropilenă) de tip meltblown. Frunzele de măslin din regiunea Antalya sunt recoltate, uscate la aer și apoi pulverizate în particule fine. Particulele de măsline măcinate sunt îndepărtate utilizând un aparat Soxhlet. Materialul extras este tratat cu un material textil pentru măști utilizând o metodă numită „impregnare-menținere-uscare", iar apoi este testat pentru activitate antibacteriană în conformitate cu standardul AATCC 147. Bacteriile Staphylococcus aureus sunt selectate pentru a reprezenta grupul gram-pozitiv, în timp ce bacteriile Escherichia coli reprezintă grupul gram-negativ. Diametrele zonelor sunt măsurate la 47 mm pentru S. aureus și 40 mm pentru E. coli. Rezultatele au indicat faptul că materialele textile infuzate cu extract de frunze de măslin, care conține oleuropeină, prezintă o eficacitate antibacteriană semnificativă. Eficacitatea medie de filtrare a bacteriilor în cazul măștilor fără aditiv de oleuropeină este de 96,7%, dar măștile cu ingredientul oleuropeină au o eficiență de 98,5%. Respirabilitatea medie a crescut de la 3,7 mm H_2O *la 4,8 mm* H_2O*. Aceste date indică faptul că performanța respirabilității a scăzut odată cu adăugarea de oleuropeină.*

Cuvinte-cheie*: frunză de măslin, activitate antibacteriană, mască facială, oleuropeină*

INTRODUCTION

Recently, there has been a notable surge in studies focused on the creation of medicinal antibacterial and antiviral fabrics [1]. This study sought to develop a face mask that establishes an efficient barrier against bacteria and viruses during and subsequent to the COVID-19 pandemic. Therefore, if the mask surface becomes contaminated, it will reduce and eliminate the viability of bacteria and viruses. Numerous prior investigations have demonstrated the antibacterial, antiviral, and antifungal properties of the compound oleuropein. The literature review indicates that oleuropein is present in high concentrations in olive leaves [2, 3].

The coronavirus (COVID-19), which originally emerged in Wuhan, China, in January 2019, has evolved into a global pandemic and has become the foremost challenge facing humanity today. Furthermore, research indicates that COVID-19 will not be the final virus to impact the globe. Consequently, humanity must contend with and manage bacteria and viruses. The significance of masks has been increasingly apparent in this conflict. The primary mask types advised for the COVID-19 pandemic are "Medical Face Mask–Surgical Mask", "FFP2/FFP3 (Filtering Face Piece) Non-vented Masks", and "FFP2/FFP3 Valve Masks". FFP2/FFP3 valveless masks and FFP2/FFP3 valve masks are predominantly utilised by healthcare professionals (table 1) [4].

Nonetheless, beyond medical applications, the utilisation of masks for public health has become an obligatory and essential precaution. The masks utilised by the public are referred to as "medical face masks" or "surgical masks". Surgical masks are composed of

Table 1

CLASSIFICATION OF MASKS [4]			
Function	**Medical face mask**	**FFP2/FFP3 Mask without valve**	**FFP2/FFP3 Valve mask**
Protecting the user	X	✓	✓
Protecting the environment	✓	✓	X
Standards	EN 14683	EN 149-A1 (EN 14683)	EN 149-A1

three distinct layers. The exterior and inner layers are composed of polypropylene non-woven fabric manufactured using the spunbond process, and the central layer is made of polypropylene (PP) non-woven fabric produced by the meltblown technique. The three layers are combined to form a mask (figure 1) [4].

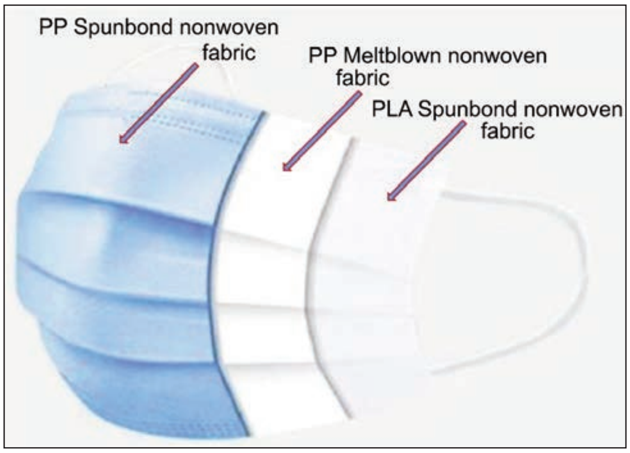

Fig. 1. Three-layer surgical mask made of PP non-woven fabric [4]

Medical face masks are categorised into three classifications: Type I, Type II, and Type III, in accordance with the EN 14683 standard (table 2). According to ASTM F 2100-07, masks fall into three categories: "low-level barrier", "medium-level barrier", and "high-level barrier" (table 2). Type-I masks, preferred by the public versus FFP-type masks, can cause skin problems like allergies, diaper rash, and acne in certain skin types and fail to provide 95% protection against viruses. Furthermore, another drawback of these masks is that they emit an odour of breath after several hours of use. The inspiration for this study stems from the design of a new mask intended to address the issues associated with "Type-I medical face masks".

Numerous studies in the literature have examined medical face masks that incorporate additives such as silver, copper, lignin, chitosan, etc. The middle layer of the mask is dipped in the solution containing lignin/copper nanoparticles for 24 hours and then dried to evaluate the antibacterial activity. The mask coated with lignin/copper nanoparticles showed no cytotoxicity and killed microbes by destroying cell membranes [6]. In another study, researchers looked at how well silver nanoparticle-sodium alginate and chitosan nanocomposite films can fight bacteria in acne treatment, and they found that these films created clear areas free of bacteria that were between 12.33 mm and 13.67 mm wide against *S. aureus, E. coli*, and *C. acnes* bacteria [7].

The mask utilised in this investigation is produced by impregnating olive leaf extract onto a three-layer fabric composed of nonwoven polypropylene materials. According to the results of the investigations, the oleuropein compound that is extracted from olive leaves is a naturally occurring chemical that possesses antifungal, antiviral, and antibacterial qualities. Studies have indicated that the oleuropein chemical offers 99% viral prevention.

Olive leaf has been utilised in traditional medicine for generations. For example, the 1800s recognised its use in combating the malaria epidemic. The American

Table 2

CLASSIFICATION OF SURGICAL MASKS ACCORDING TO EN 14683 AND ASTM F 2100 [5]						
Test	**EN 14683**			**ASTM F2100**		
	Type I	**Type II**	**Type IIR**	**Level 1**	**Level 2**	**Level 3**
Bacterial filtration efficiency (%)	≥95	≥98	≥98	≥95	≥98	≥98
Differential pressure mm (H_2O/cm^2) (Pa/cm^2)	<3.0 <29.4	<3.0 <29.4	<5.0 <49.0	<4.0 <39.2	<5.0 <49.0	<5.0 <49.0
Sub-micron particulate filtration efficiency at 0.1 micron (%)	Not required	Not required	Not required	≥95	≥98	≥98
Splash resistance/Synthetic blood resistance (mmHg pass result)	Not required	Not required	120 (16.0 kPa)	80	120	160
Flame spread	Not required	Not required	Not required	Class 1	Class 1	Class 1
Microbial cleanliness (cfu/g)	≤30	≤30	≤30	Not required	Not required	Not required

Cancer Research Institute has declared that olive leaf is the preeminent natural antibacterial and antiviral plant of the 21st century [8, 9]. Moreover, numerous studies indicate that oleuropein may be effective against HIV, parainfluenza type 3 virus, herpes mononucleosis, hepatitis virus, rotavirus, bovine rhinovirus, canine parvovirus, and feline leukaemia virus [10, 11].

The American Food and Drug Administration (FDA) has sanctioned the use of 29 medications containing oleuropein in the battle against the coronavirus. 97% of the world's olive trees are in the Mediterranean region, specifically in Spain, Italy, Greece, Türkiye, and Tunisia, as well as in nations like Portugal and Syria. The olive tree proliferates naturally in the maquis vegetation of these nations and has extensive distribution. In Türkiye, the extraction of oleuropein from olive leaves and its subsequent use are crucial for scientific research [2]. In all these nations, solely the fruit of olive trees is collected.

Nonetheless, its leaves possess significant value and can be transformed into a product that yields economic revenue. The leaves, a by-product, are generated during the trimming of the trees, the harvesting of olives, and the cleaning and blending operations involved in olive oil production.

Oleuropein, derived from olive leaves, has been researched for its applications and utilised as a medicinal agent in the medical profession.

Nonetheless, the utilisation of oleuropein on textile materials for its antiviral, antibacterial, and antifungal characteristics has not been investigated too far. Phenolic compounds present in olive leaves include secoiridoids (oleuropein, dimethyloleuropein, verbascoside, ligtroside, and oleuroside), phenolic acids and their derivatives (vanillic acid, caffeic acid, and vanillin), phenolic alcohols (tyrosine and hydroxytyrosol), flavones (lutozeoline-7-glycoside, diosmetin-7-glycoside, luteolin, and diosmetin), flavonoids (quercetin, isorhamnetin, and rutin), and flavanols (catechin and gallocatechin) [12, 13].

Oleuropein is the predominant phenolic component found in olive leaves. This is succeeded by hydroxytyrosol, luteolin, apigenin flavone-7-glycosides, and verbascoside. Analysis of oleuropein content in olive leaves reveals that the concentration in the dried leaves of 14 olive trees ranged from 9.0% to 14.3% [14].

MATERIAL AND METHOD

Procurement of olive leaves and oleuropein compound

The oleuropein utilised in the project is sourced from olive leaves harvested from olive trees in the Mediterranean region. Subsequent to the drying of these leaves, they are subjected to extraction via a Soxhlet apparatus. The extracted sample is analysed using the HL-PC instrument to quantify its oleuropein content.

Provision of mask fabric

Non-woven materials are typically favoured for surgical masks. These fabrics are favoured because of their superior performance in bacteria filtering and air permeability compared to woven materials. The primary raw material for nonwoven fabrics is mostly polypropylene (PP) [15]. The masks have an inner and outer layer made from PP nonwoven fabric produced by the spunbond process, and the middle layer is made from PP nonwoven fabric created using the meltblown technique. The fabrics for the mask in the project are sourced from a mask manufacturing company.

Identification of bacteria

At the outset, it is important to emphasise the following points. The initiative aims to utilise bacteria from the respiratory tract. Due to the hazardous nature of testing these bacteria and the requirement for authorisation, a generalisation is employed, utilising *S. aureus* and *E. coli* to symbolise gram-positive and gram-negative bacteria, respectively. The personnel of Mehmet Akif Ersoy University Microbiology Laboratory facilitated the bacterial testing conducted. Research on oleuropein indicates its efficacy against several fungi, bacteria, and viruses. Oleuropein has demonstrated antiviral efficacy against mononucleosis herpes, hepatitis viruses, rotaviruses, bovine viruses, parvoviruses in canines, and leukaemia viruses in felines [16].

Numerous taste compounds exhibit antibacterial properties against *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Candida utilis*, and *Aspergillus niger* [17]. Olive leaf exhibits inherent resistance to microbes and insect infestations [18]. Initial documentation of its therapeutic properties as an antipyretic dates back to 1854, and subsequent reports of its antihypertensive and antibacterial actions follow [19].

Certain research indicates that olive leaves are abundant in phenolic compounds and α-tocopherol. The main phenolic compounds found in olive leaves are oleuropein, luteolin, luteolin-7-glucoside, apigenin, apigenin-7-glucoside, hydroxytyrosol, chlorogenic acid, p-coumaric acid, and rutin (quercetin-3-rutinoside) [20]. Research indicates that phenolic compounds in olive fruits and leaves exhibit inhibitory and retarding effects on microbial development.

Additionally, olive leaf extract possesses antioxidant properties [19] and serves as a potential source of antifungal agents, making it applicable as an additive in both the food and pharmaceutical industries [18].

Acquisition of olive leaf extract

Olive leaves are disseminated and desiccated at ambient temperatures for 15 days. The desiccated leaves are pulverised in a grinder and reduced to diminutive fragments. The leaves, reduced into minute fragments, are weighed on a precision scale and prepared for extraction (figure 2).

Fig. 2. Dehydrating and pulverising the olive leaves

The literature review revealed that the solid-liquid extraction process is employed to extract some components from natural plants, utilising an apparatus known as the “Soxhlet Extractor”. Consequently, the Soxhlet Extractor is employed to extract the oleuropein compound from pulverised olive leaves (figure 3).

Fig. 3. Extraction of olive leaves with Soxhlet device (Burdur Mehmet Akif Ersoy University)

Extraction is conducted using a Soxhlet apparatus as outlined below. The dried leaves, chopped into minute fragments, are weighed and placed into the cellulose extraction cartridge. The cartridge is, moreover, situated within the extraction arm. A chemical solvent is added to the glass flask and subsequently evaporated using a heater. The evaporating solvent ascends to the reflux via the extraction column. The solvent, concentrated in the rear cooler, flows to the extraction arm, dissolving the substance in the cartridge before returning to the glass flask. This technique is perpetually reiterated to finalise the extraction. The isolated compound is gathered in the flask. The residual solvent in the flask is eliminated, yielding the component to be extracted in its pure form [12].

Quantification of oleuropein concentration in olive leaf extract using High-Performance Liquid Chromatography (HPLC)

The concentration of oleuropein in the extract is quantified using the HPLC apparatus at Süleyman Demirel University Laboratory. 10.7 mg of the extract is mixed with 1 ml of methanol (figure 4), then diluted 20 times, and tested with the HPLC machine to measure the amount of oleuropein. The characteristics of the Shimadzu HPLC device are Detector: DAD detector (λmax = 278 nm), Autosampler: SIL-10ADvp, System Controller: SCL-10Avp, Pump: LC-10ADvp, Degasser: DGU-14A. Column oven: CTO-10Avp Agilent Eclipse XDB-C18 Column (250 × 4.60 mm) 5 microns Mobile phase: A: 3% acetic acid; B: methanol. Flow Rate: 0.8 ml per minute.

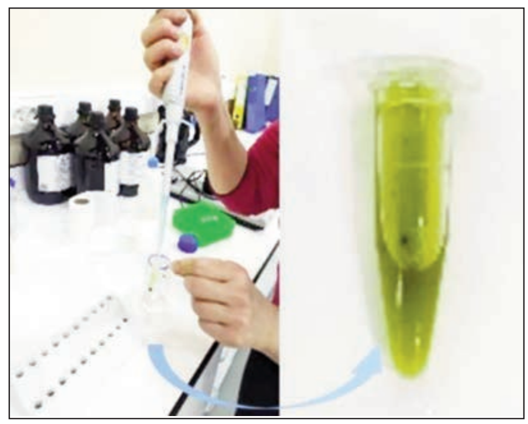

Fig. 4. Dissolving the extract in methanol

Absorption of olive leaf extract in nonwoven fabrics

The application of oleuropein extract to the three-layer mask fabric is conducted using the “soak-dry-hold” method [21]. Methanol serves as a solvent in the impregnation procedure. A 5% citric acid solution is utilised to bind the 20% extract to the mask fabric. The solution’s temperature is set to 35 °C and pH 5. The impregnation process is conducted for 25 minutes utilising a hot magnetic stirrer at a liquor ratio of 1:20. The solution-saturated fabric is subjected to 70% pressure in a laboratory squeezing machine to eliminate excess solution. The fabrics are maintained in the oven at 40 °C for a duration of 10 minutes for drying purposes.

Evaluation of antibacterial efficacy of extract-infused fabric

The antibacterial activity of the fabric infused with olive leaf extract was evaluated using the AATCC 147 test technique. The AATCC 147 diffusion agar method is a widely utilised standard for assessing the

Table 3

CLASSIFICATION OF SURGICAL MASKS ACCORDING TO ASTM F 2101 AND MIL-M-36954 C STANDARDS [23]			
Scale	**Level 1 barrier**	**Level 2 barrier**	**Level 1 barrier**
MIL-M-36954 C: ΔP (Breathability)	<4 mm H_2O	<5 mm H_2O	<5 mm H_2O
ASTM F2101: BFE (Filtration 3 μm)	≥% 95	≥% 98	≥% 98

antibacterial efficacy of textile fabrics. In accordance with the AATCC 147 standard, pre-prepared bacterial concentrations are introduced into the medium, followed by the placement of fabric samples with a diameter of 25 mm. Following a 24-hour incubation of the sample fabrics at 37 °C, the efficacy of the sample fabric is assessed in millimetres by measuring the diameter of the inhibitory zone surrounding the fabric. The agar diffusion method, a quantitative approach, assesses the antibacterial activity of treated fabrics, allowing for evaluations of their effectiveness [22].

*Escherichia coli (*ATCC 35150) and *Staphylococcus aureus* (ATCC 25923) are cultured in petri dishes, after which nonwoven textiles, each measuring 25 mm in diameter, are positioned in containers. The effectiveness of the antibacterial activity is measured in millimetres by looking at the size of the clear area around the fabric after the nonwoven materials were kept at 37 °C for 24 hours.

Bacterial filtration efficiency assessment of a mask

Bacterial filtration efficiency (BFE) quantifies a material's resistance to bacterial infiltration. The bacterial filtering efficiency test is conducted utilising the GT-RAO2 tester. An aerosol comprising microorganisms of a specific size (e.g., *Staphylococcus aureus*) is discharged towards the mask. The dimensions of the bacterial particles typically measure approximately 3 μm. The test is typically conducted at an airflow rate of 28.3 l/min. A segment of the aerosol is transmitted via the mask, and the mask's filtering efficacy is assessed. The results are expressed as a % efficiency, indicating the fabric's capacity to withstand bacterial penetration. Elevated values in this assessment signify enhanced barrier efficacy. The ASTM F2101 standard test protocol defines it as the proportion of particles filtered out by the mask. This testing method is specifically formulated to assess the bacterial filtration efficiency of medical face masks, utilising *Staphylococcus aureus* as the challenge organism. The utilisation of *S. aureus* is predicated on its clinical significance as a predominant cause of nosocomial infections. The highest filtration efficiency ascertained by this technology is 99.9% [23].

Mask breathability assessment

The breathability assessment is conducted in accordance with MIL-M-36954 C. This assessment evaluates a face mask's resistance to airflow. A regulated airflow is sent through the mask, and the pressure differential (ΔP) is monitored before and after the passage. The pressure differential is divided by the sample's surface area (in cm^2). Reduced breathing resistance signifies an enhanced comfort level for the user.

The classification of surgical masks is presented in table 3 in accordance with ASTM F 2101 and MIL-M-36954 C standards.

RESULTS AND DISCUSSION

The measurement conducted using the HPLC instrument indicates that the concentration of oleuropein in 1 gram of extract is 106.9 mg/g. The olive leaf utilised in the experiment has 10.6% oleuropein. The chromatogram of the sample is presented in figure 5. The graph verifies that the apex at the 66th minute corresponds to oleuropein.

Figure 6 presents the widths of zones created in the fabric as a consequence of the test conducted in

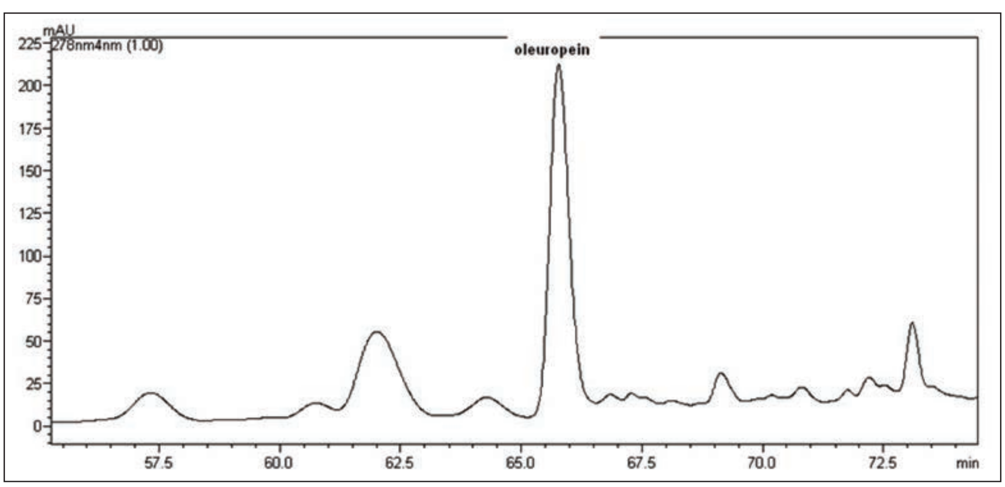

Fig. 5. The chromatogram produced by the HPLC apparatus

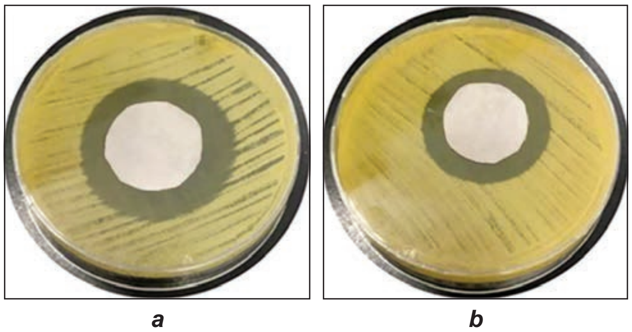

Fig. 6. The zone diameter produced by the mask containing 20% extract on: *a* – *S. aureus* bacteria; *b* – *E. coli* bacteria

accordance with the AATCC 147 diffusion agar method. The zone diameter for *S. aureus* bacteria is illustrated in figure 6, *a*, while the zone diameter for *E. coli* bacteria is depicted in figure 6, *b*. A 47 mm zone of inhibition is observed against *S. aureus*, while a 40 mm zone is noted against *E. coli*.

The findings indicate that olive tree leaves in the Mediterranean region contain approximately 10.6% oleuropein. The absorption of the olive leaf extract into the three-layer mask fabric and its adherence to the fabric are effectively achieved utilising the "soaking-drying" approach, which is both straightforward and cost-effective.

The study also found that olive leaf extract is particularly effective against *S. aureus* and *E. coli* bacteria. The study's tests indicate that fabric infused with olive leaf extract exhibits superior efficacy against *S. aureus* bacteria, with a measured inhibition zone diameter of 47 mm. The inhibition effect against *E. coli* bacteria is found to be inferior to that against S. aureus bacteria, with a measured zone diameter of 40 mm. This effect is believed to be associated with the cell wall of *E. coli* bacteria.

These results correspond with the literature. A study indicates that oleuropein derived from olive leaves exhibits significant efficacy against *S. aureus* bacteria, with an inhibitory diameter of 30.18 mm [24]. A different investigation revealed that a combination of phenolic compounds from olive leaves exhibited superior antibacterial action compared to individual phenolic compounds examined in isolation [25]. Consequently, they exhibited the antibacterial properties of phenolic compounds found in olive leaves.

The bacterial efficiency test is performed on masks with and without oleuropein added, following the ASTM F2101 standard, using ten repetitions to find the average results. Table 4 presents the mean results. Upon examination of the values, it is observed that the average bacterial filtration effectiveness of masks without an oleuropein additive is 96.7%; however, this number increases to 98.5% in masks with an oleuropein additive. The oleuropein addition enhanced the bacterial filtering performance of the masks by 2.2%. Upon examination of the breathability values of the masks, it is evident that the addition of oleuropein diminishes the breathability of the mask. This phenomenon is believed to arise from the

Table 4

BACTERIAL EFFICIENCY AND BREATHABILITY RESULTS		
Type of mask	**Bacterial efficiency average (%)**	**Breathability average (mm H_2O)**
Non-additive mask	96.7	3.7
Mask with oleuropein	98.5	4.8

oleuropein additive closing the pores between the fibres, which aligns with existing literature.

CONCLUSION

The HPLC test conducted on olive leaves from olive trees in Antalya province, Türkiye, revealed a high oleuropein concentration of approximately 10.6%. In line with the literature, oleuropein is identified as the predominant phenolic compound in olive leaves.

The extract derived from olive leaves has been applied to the fabric by the "soak-hold-dry" technique and has effectively been integrated into the fabric. The employed procedure is remarkably straightforward and cost-effective. The olive leaves utilised in the study are wastes generated post-harvest. Thus, the waste olive leaves not only have been converted into a high-value product, but also the creation of environmental waste has been prevented.

Antibacterial effectiveness assessments have been conducted on oleuropein additive textiles against *S. aureus* and *E. coli* bacteria in accordance with the AATCC 147 standard. The test findings indicated that the textiles exhibited a zone diameter of 40 mm against *E. coli* bacteria and a zone diameter of 47 mm against *S. aureus* bacteria. It is determined that fabrics treated with oleuropein exhibit better effectiveness against *S. aureus* bacteria.

E. coli bacteria are widely recognised in the field of medicine. This bacterium is a type of gram-negative bacterium that proliferates at body temperature, leading to various illnesses and exhibiting infectious properties. *S. aureus* is a gram-positive bacterium that induces several illnesses in humans. These bacteria are prevalent because of their resilience to fluctuating climatic conditions and are predominantly located in wounds of the nose, throat, and skin. Direct contact with air and surfaces can spread these bacteria. Using oleuropein additives in medical textiles will reduce the spread of these bacteria and help prevent diseases caused by them. The findings have indicated that the oleuropein addition enhanced the bacterial efficiency value of masks by 2%. According to ASTM F2101 standards, a non-additive mask has exhibited an average bacterial effectiveness of 96.7%, but the mask including the oleuropein additive has an average bacterial efficiency of 98.5%.

Nevertheless, according to the MIL-M-36954 C standard, the breathability test conducted on the oleuropein additive masks has demonstrated an increase from 3.7 mmH_2O to 4.8 mmH_2O. This result shows a reduction in the mask's breathability. This situation has arisen because the contribution of oleuropein has blocked the gaps between the fibres. Thus, the air permeability of the fabric structure made of fibres has decreased. Nonetheless, the value attained falls within acceptable parameters. ASTM standards categorise masks into three levels: Level 1 – Low barrier protection, Level 2 – Moderate barrier protection, and Level 3 – Maximum barrier protection. Upon examining the data from this study and comparing it with ASTM requirements, we can see that the values of the 'oleuropein additive masks' fall within the Level 2 Barrier classification. It is generally advised to utilise Level 1 Barrier masks for daily protection at a specified distance and in situations with a low risk of bacterial contamination, whereas Level 2 Barrier masks are indicated for scenarios with a high risk of contamination. The result indicates that oleuropein additive masks are suitable for daily use or surgical procedures with a heightened risk of contamination.

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