The Potential Uses of Olive Leaf Extracts in Various Areas

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Abstract

Olive leaves have been used in traditional remedies for centuries. In this study, the antimicrobial activity of the leaf extracts from Ayvalık Yaglik and Manzanilla varieties against food and clinical test microorganisms, fish pathogens and human milk originated lactic acid bacteria (LAB) and also sun protection factor (SPF) were investigated. The results of the disc diffusion assay indicated that the extracts showed antimicrobial activity against the tested microorganisms with inhibition zones from 8.52 mm to 19.36 mm. Most of the olive extracts did not show antibacterial activity on the LAB tested. Therefore, the extracts that has no inhibitory activity can be used together with LAB in various industries. The SPF values of the extract and the extract+cream mixture were between 0.05 and 16.46. The results indicated that the olive leaf extracts may be used as natural sunscreen additive in the cosmetics industry. In addition, the extracts may be used as natural antimicrobial substance in feed, food and pharmaceutical products as an alternative to chemical preservatives.

Keywords: Antimicrobial, Extract, Natural Preservatives, Ultra Violet, Cream

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INTRODUCTION

Olive is one of the most suitable crop for Mediterranean countries. Olive and its by-products are one of the main foods of the Mediterranean diet (Boudhrioua et al., 2008; Pereira et al., 2007). Synthetic preservatives and antioxidants provide high antimicrobial and antioxidant properties, but most of these chemical preservatives are toxic and have many side effects (Raymond et al., 2009; Chen et al., 2018). Currently, there is an increasing demand for high-quality products with low synthetic chemicals and long shelf life. Therefore, there is an urgent need to develop foods, cosmetics and medicines that used natural substances to provide self-preservation and/or protection from microbial growth. The antibacterial properties of plants have been extensively studied (Ben-Othman et al., 2020). Olive leaf can be obtained during pruning and has both antibacterial and antioxidant properties (Lee & Lee, 2010). As a result, the conversion value products have the potential to become the sustainable and environmentally friendly product that replaces current disposal (Lafka et al., 2013).

Diseases caused by food and clinical microorganisms are an important problem for public health. For example, *Escherichia coli* can be found in undercooked ground beef, raw milk, raw products, grains, as well as soft cheese made from water, unsterilized milk, and fruit juice (Poudyal et al., 2010; Karygianni et al., 2014; Liu et al., 2017). Salmonella usually infects humans through water and food. As a result of several studies, the antibacterial activity of olive leaf extract (OLE) against *Salmonella typhimurium* has been reported (Pereira et al., 2007; Erdogan & Turhan, 2012).

Staphylococcus aureus can cause a variety of illnesses. It can cause a variety of skin infections, including abscesses, boil, carbuncle, cellulitis, folliculitis, impetigo, acne, and burned skin syndrome (Ayana & Turhan, 2009). *Bacillus cereus* produces toxins and is common in food poisons (Ahmed et al., 2014). OLE suppressed bacterial growth (Markin et al., 2003). *Candida albicans* is a common pathogenic species that primarily affects people with immunodeficiency (Poudyal et al., 2010; Brahmi et al., 2012). The antibacterial activity of olive leaf extract has been reported to have a significant effect on the suppression of *C. glabrata* (Sudjana et al., 2009).

The healthy development of fish in aquaculture in developed and developing countries is economically and ecologically important (Suez 2019). Fish pathogens that cause disease outbreaks are considered a major threat to aquaculture (Menanteau-Ledouble et al., 2018). *Aeromonas hydrophila* and *Yersinia ruckeri* as fish pathogens causes hemorrhagic septicemia syndrome and leads to increased mortality in aquaculture (Onuk et al., 2015; Brown & Dawson, 2015).

Olive leaf has many effects such as antimicrobial (Korukluoğlu et al., 2010), antioxidant (Benavente-Garcia et al. 2000), hypocholesterolemic (Jemai et al., 2009), cardioprotective (Nekooeian et al., 2014). These beneficial effects of olive leaf are associated with the phenolic compounds in its structure (Vogel et al., 2015).

Today, the demand for probiotic-containing products that support the immune system is increasing. Probiotics are live microorganisms that have beneficial effects on host health by regulating the gastrointestinal tract (Fuller 1989). Lactic acid bacteria (LAB), which are frequently used in biotechnological studies, are of great importance as they are a group of microorganisms used extensively in the production of fermented products, industrial food fermentation, dairy in, industry and food technology. LAB is of great importance due to its use in the health and sensory properties of food products and animal feeds. Recently, scientific interest in LAB has increased due to its importance in food production and various studies have been conducted on the subject (Schaafsma 2008).

Although the sun is a source of life for all living things, it also affects the daily life of living things on earth (Matsui et al., 2009). Exposure to UV radiation can cause harmful effects such as dryness, wrinkles, pigment abnormalities and skin cancer (Nichols & Katiyar 2010). It is recommended to use sunscreen products to protect against the harmful side effects of UV rays. Sunscreen agents have a sun protection factor (SPF) value, which is defined as the ratio of the minimum erythema dose to the sunscreen agent (Riva et al., 2006). Plant extracts obtained by different methods can prevent the acceleration of some transcription factors caused by UV rays in skin cells, and plant extracts may contain bioactive substances that can help prevent harmful rays from the sun and protect the skin (de Oliveira et al., 2016). The purpose of this study is to investigate the antimicrobial effects of olive leaf extracts obtained from Ayvalık Yaglik (AY) and Manzanilla (M) varieties on food/clinical microorganisms and fish pathogens to determine its usage potential as a natural additive in the food and feed industries. In addition, it was aimed to determine the potential use of the olive extracts together with LAB as a natural additive in the food and health industries. The SPF value of the various olive leaves extracts was also purposed to determine for potential use as a cheaper and safer alternative to sunscreens containing harmful chemicals in the cosmetic industry.

MATERIAL and METHOD

Antimicrobial activity

Extract preparation

The olive leaves samples of AY and M varieties were obtained from Izmir Olive Research Institute in September 2019. The plant material was dried in an airy environment without sunlight at room temperature. The dried olive leaves are grounded. In extraction, 10 g powder from olive leaves were extracted with 30 ml of ethanol (96%), methanol, and acetone using a sonicator device (Hielscher) on ice in 3 repetitions in 10 minutes. After extraction, the solvents were evaporated. Then, the obtained solutions were sterilized by 0.45 µm Millipore filters. The extracts were kept at 4°C under dry conditions until they were used.

Determination of Antimicrobial Activity

Disc Diffusion Assay

The antimicrobial activity of olive leaf extracts was determined with disc diffusion assay. *E. coli* O157:H7, *B. subtilis* RSKK 244, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *B. cereus* RSKK 863, *E. faecalis* ATCC 29212, *S. sonnei* Mu:57, *Yersinia enterocolitica* ATCC 1175, *E. coli* ATCC 35218, *S. enteritidis* RSKK 171, *A. hydrophila* were cultured in Nutrient/broth (NB) at 37°C. *L. monocytogenes* ATCC 7644, *S. agalactiae* Pas. Ins. 55118, *L. garvieae*, *Y. ruckeri* were growth in Tryptic Soy Broth (TSB)/Agar at 37°C. *V. anguillarum* A4, *V. anguillarum* M1, *V. alginolyticus* in Tryptic Soy Broth/NaCl medium at 25°C, *C. albicans* ATCC 10231 and *C. glabrata* RSKK 04019 in Yeast Extract Peptone Dextrose (YPD)/Agar at 30°C'de, *L. gasseri* MA-1, *L. gasser*i MA-2, *L. gasseri* MA-6, *L. fermentum* MA-7, *L. fermentum* MA-8, *Lactobacillus delbrueckii* MA-9 in De Man, Rogosa and Sharpe (MRS) at 37°C were cultured. The active cultures of the test microorganisms were washed twice with physiological saline. Then, their concentrations were adjusted to 0.5 McFarland and inoculated into the appropriate solid medium. Sterile discs with a diameter of 6 mm were placed on the inoculated growth medium. Then, 20 µL of the extracts of AY and M varieties were dropped onto the discs. Petri dishes were incubated at appropriate temperatures for 24 h. At the end of the incubation period, the zones around the discs were measured and recorded. In the study, Ampicillin (AM, 10 µg/disc), Kanamycin (K, 30 µg/disc) and Fluconazole (FCA, 25 µg/disc) were used as the control. All experiments were performed in triplicate.

Determination of Minimum Inhibition (MIC) and Minimum Bactericidal or Fungicidal (MBC or MFC) Concentrations with the micro-dilution method

MIC and MBC or MFC values of the extracts were determined by micro-dilution assay against test microorganisms. Test microorganisms at 0.5 McFarland concentration were added to each tube containing the extract and medium and mixed gently. Then, the tubes containing the mixture were incubated at appropriate temperatures for 24 h. After the incubation, the concentration at which there was no growth after incubation was determined as the MIC value. Then, the samples were taken from the tubes, and spot-dropped was made on the specific agar medium and incubated at the appropriate temperatures. At the end of the incubation period, the concentrations of the extracts that prevent the growth of microorganisms on the solid medium were recorded as MBC or MFC values.

In-vitro sun protection factor (SPF)

SPF values of leaf extracts from AY and M varieties was determined in vitro. The extracts were prepared in triplicate in ethanol (96%) at a concentration of 2 µg/µL. The homogeneous mixture was measured in the spectrophotometer (Beckman Coulter) at 5 nm intervals between 290 nm and 320 nm wavelength. The values were calculated using the Mansur equation (Mansur et al. 1986) as below.

$$\mathbf{SPF = CF\ x}\ \sum_{290}^{320} \boldsymbol{EE}(\boldsymbol{\lambda}) \text{x}\ \boldsymbol{I}(\boldsymbol{\lambda}) \text{x}\ \boldsymbol{Abs}\ (\boldsymbol{\lambda}) \quad (1)$$

CF = Correction Factor (= 10)

abs (λ) = Wavelength absorbance of extracts (λ).

I (λ) = Intensity of sunlight at the wavelength (λ)

EE(λ) = Erythemetogenic effect radiation wavelength (λ)

Sun protection factor of extract and cream mixture

The developed modified method using Imam et al. (2015) and Bambal et al. (2011) protocols was used to determinate SPF value of extract and cream mixtures. For each extract, 1 g of cream was weighed separately and 0.5 g of each extract was added to this cream. The mixture was made up to 10 g with distilled water. 0.1 g of this prepared mixture was taken into another tube and 10 ml was completed with ethanol (40%). It was then sonicated for 5 minutes. After this mixture was filtered through No:1 Whatman filter paper, 0.5 ml was taken into another tube and made up to 5 ml with ethanol. 0.5 ml of the mixture was taken and the volume was completed to 2.5 ml. The mixtures adjusted as 2.5 ml, 5 ml and 10 ml were measured in 3 repetitions in a spectrophotometer at 5 nm intervals in the wavelength range of 290 nm - 320 nm. The values were calculated using the Mansur equation (Mansur et al. 1986).

RESULTS and DISCUSSION

Microbial resistance to antibiotics is increasing day by day. There are many new studies on alternative solutions to reduce the negative effects of antibiotics on human health. The studies show that olive leaves have very good biological activity (Goreishi & Shahrestani, 2009; Khalil et al., 2014). In the current study, the antimicrobial activity of leaf extracts from AY (AYOL) and M (MOL) against food and clinical microorganisms and fish pathogens and LAB were assessed using disc diffusion assay. Micro-dilution assay for determination MIC, MBC, and MFC values of the extracts also was used. The results are presented in Table 1-5.
The results indicated that both AYOL and MOL inhibited the growth of all tested microorganisms with inhibition zones ranged between 8.52 - 19.36 mm (Table 1-2). MIC and MBC or MFC concentrations of AYOL and MOL extracts varied in same ranges as 10 - 40 µg/µL and 10 - 80 µg/µL (Table 3-5). All AYOL extracts inhibited the growth of all the tested yeasts. AYOL methanol extract showed the highest inhibition zone diameter for both *S. sonnei* MU:57 (19.07 mm) and *Y. enterolitica* ATCC 11175 (17.90 mm) among food and clinical pathogens. FCA (25 µg) showed no inhibitory activity against *C. albicans* ATCC 10231. AYOL extracts showed high inhibition zone diameter (12.99 mm) on *C. glabrata* RSKK 04019. AYOL methanol extract against *V. angillarum* M1 (fish pathogen) showed high inhibition zone activity (14.42 mm) (Table 1). AYOL ethanol, acetone, and methanol extracts did not inhibit the growth of five LABs. The methanol extract showed only inhibition activity (13.90±0.7) against *L. gasseri* MA-2. It is predicted that if the olive extract concentrates are in the appropriate concentrate range, they will not prevent the growth of LAB originated from human milk (Table 1).

Table 1. Inhibition zone diameters of Ayvalık Yaglik variety olive leaf extracts and antibiotics (mm ± SD).

Strains	**E**	**M**	**A**	**AM**	**K**	**FCA**
Food and Clinical test Microorganisms						
E. coli O157:H7	12.72±1.5	12.46±0.9	9.83±02	17.76±0	19.33±0.4	-
B. subtilis RSKK 244	13.02±0	14.87±0.8	12.68±0.2	30.27±0.9	19.36±0.1	-
B. cereus RSKK 863	11.30±1.2	11.37±0.3	10.99±0.6	16.81±0.2	12.97±0.3	-
S. aureus ATCC 25923	11.60±0.4	13.12±0.8	11.81±0.5	21.04±08	19.91±0.5	-
P. aeruginosa ATCC 27853	12.10±0.3	11.21±0.6	10.90±0.2	23.53±0.6	20.37±0.2	-
E. coli ATCC 35218	11.19±0.1	11.69±0.1	11.23±0.71	18.75±.0.7	14.11±0.1	-
Y. enterolitica ATCC 11175	16.71±0.5	17.90±1.7	15.48±0.5	-	18.10±5.7	-
L. monocytogenes ATCC 7644	16.36±0.6	17.05±1	15.92±0.2	29.57±0.1	25.40±1.3	-
S. sonnei MU:57	14.24±0.7	19.07±0.5	15.50±0.6	13.48±1.4	11.08±0.8	-
E. faecalis ATCC 29212	17.32±1.3	17.41±0.6	14.89±1.2	24.02±0.3	13.48±1.4	-
S. enteritidis RSKK 171	12.63±0.5	15.67±0.6	11.50±0.9	14.02±0.3	15.48±1.4	-
C. albicans ATCC 10231	12.10±0.4	12.75±0.7	11.69±0.21	-	-	-
C. glabrata RSKK 04019	12.18±1	12.99±0.2	9.75±0.6	-	-	20.35±0.1
Fish Pathogens						
S. agalactia Pas.Ins. 55118	12.67±0.6	13.39±1.8	12.51±1.1	37.46±0.12	9.89±0.21	-
V. angillarum A4	13.60±1.1	12.16±0.3	11.06±0.9	9.40±0.11	13.76±0.03	-
V. angillarum M1	13.03±0.5	14.42±0.6	12.71±1.5	9.02±0.04	14.81±0.02	-
V. alginolyticus	10.10±0.2	10.33±1.4	8.81±0.2	13.57±0.09	15.05±0.11	-
Y. ruckeri	11.36±0.5	12.44±0.8	9.42±0.7	32.30±0.15	17.54±0.07	-
A. hydrophila ATCC 19570	11.05±0.6	12.33±0.3	10.45±0.9	11.20±0.06	16.16±0.05	-
L. garvieae	9.98±0.9	11.13±0.2	10.33±0.7	33.10±0.12	23.05±0.14	-
LAB						

L. delbrueckii MA-9	-	-	-	23.73±0.75	-	-
L. gasseri MA-1	-	-	-	23.18±2.61	-	-
L. gasseri MA-2	-	13.90±0.7	-	24.31±0.63	-	-
L. fermentum MA-8	-	-	-	28.68±1.29	-	-
L.fermentum MA-7	-	-	-	28.42±1.00	-	-

E: Ethanol, M: Methanol, A: Acetone, AM: Ampicillin (10 µg), K: Kanamycin (30 µg), FCA: Fluconazole (25 µg), -: No Activity, LAB: Lactic Acid Bacteria

Among food and clinical pathogens, MOL methanol extract showed high zone of inhibition activity on *S. enteritidis* RSKK 171 (19.36 mm). All MOL extracts inhibited the growth of Candida species. MOL ethanol extract showed high inhibition zone activity for fish pathogens on both *V. angillarum* A4 (13.37 mm) and *V. angillarum* M1 (13.61 mm) (Table 2). The MOL extracts except for ethanol and acetone extracts against *L. gasseri* MA-1 and methanol extract against *L. gasseri* MA-2 did not inhibit the growth of LAB tested. The fact that the extracts do not inhibit the development of the tested LAB indicates that the extract and this probiotic candidate LAB can be used together in the pharmaceutical, food and feed industries.

Oleuropein found in olive fruit and leaves has been reported to inhibit the growth rate of microorganisms (Sudjana et al., 2009; Lee & Lee, 2010). It has been determined that oleuropein and its degradation products have inhibitory effects on *E. coli, E. faecalis, B. cereus, S. aureus, S. enteritidis, V. alginolyticus, C. glabrata* and *C. albicans* (Furneri, 2002). In a study, the antimicrobial properties of phenolic compounds in the aqueous extracts of powdered olive leaves were investigated. It is reported that the inhibitory effect of various concentrations of the extract on microorganisms is found as *B. cereus ~ C. albicans > E. coli > S. aureus > P. aeruginosa > B. subtilis* (Pereira et al., 2007). In another study with the fruit methanol extract of Alcaparra olives was tested on *B. cereus, P. aeruginosa, B. subtilis, S. aureus, E. coli* and *C. albicans* responsible for human gastrointestinal and respiratory tract infection. The olive extract at a concentration of 50 mg/mL is resistant to *C. albicans* and sensitive to other tested microorganisms (Sousa et al., 2006).

In the study investigating the antimicrobial activity of commercially available olive leaf extract against test microorganisms, they found that the extract showed the highest antimicrobial activity against *S. aureus* (Sudjana et al. 2009). In a study investigating the antimicrobial effect of olive leaf extract obtained with 80% ethanol against *L. monocytogenes, E. coli* O157:H7 and *S. enteritidis*, it was found that olive leaf extract at a concentration of 62.5 mg/mL inhibited the growth of test microorganisms (Liu et al., 2017).

Table 2. Inhibition zone diameter of Manzanilla variety olive leaf extracts and antibiotics (mm ± SD).

Strains	**E**	**M**	**A**	**AM**	**K**	**FCA**
Food and Clinical Test Microorganisms						
E. coli O157:H7	12.25±0.3	12.53±09	11.23±0.3	17.76±0	19.33±0.4	-
B. subtilis RSKK 244	14.83±03	16.06±0.3	14.34±0.2	30.27±0.9	19.36±0.1	-
B. cereus RSKK 863	11.02±0.3	11.67±0.6	10.98±0.4	16.81±0.2	12.97±0.3	-
S. aureus ATCC 25923	11.86±0.2	12.37±1.2	11.21±0.1	21.04±08	19.91±0.5	-
P. aeruginosa ATCC 27853	11.71±0.1	12.31±0.2	11.47±0.2	23.53±0.6	20.37±0.2	-
E. coli ATCC 35218	12.24±0.1	12.45±0.2	12.07±0.4	18.75.0.7	14.11±0.1	-
Y. enterolitica ATCC 11175	15.48±0.8	17.92±0.9	15.16±0.8	-	18.10±5.7	-
L. monocytogenes ATCC 7644	17.63±0.9	18.05±0.2	13.57±0.7	29.57±0.1	25.40±1.3	-
S. sonnei MU:57	17.46±1.3	17.57±0.6	14.90±0.8	13.48±1.4	11.08±0.8	-
E. faecalis ATCC 29212	17.18±0.4	18.58±0.8	14.53±1.1	24.02±0.3	13.48±1.4	-
S. enteritidis RSKK 171	13.22±0.2	19.36±1.5	11.56±0.3	14.02±0.3	15.48±1.4	-
C. albicans ATCC 10231	13.37±0.7	12.67±0.1	10.48±0.5	-	-	-
C. glabrata RSKK 04019	11.45±0.6	11.92±0.5	10.53±0.3	-	-	20.35±0.1
Fish Pathogens						
S. agalactia Pas.Ins. 55118	12.93±0.3	12.33±0.4	10.75±0.2	37.46±0.12	9.89±0.21	-
V. angillarum A4	13.37±0.5	10.84±0.5	10.55±0.6	9.40±0.11	13.76±0.03	-
V. angillarum M1	13.61±0.6	10.28±0.4	9.02±0.4	9.02±0.04	14.81±0.02	-
V. alginolyticus	11.12±0.5	10.30±0.6	8.52±0.4	13.57±0.09	15.05±0.11	-
Y. ruckeri	12.20±1.2	10.72±1.1	9.25±0.6	32.30±0.15	17.54±0.07	-
A. hydrophila ATCC 19570	12.10±1.6	11.66±1.3	11.80±0.5	11.20±0.06	16.16±0.05	-
L. garvieae	12.32±0.2	11.39±0.8	8.53±0.2	33.10±0.12	23.05±0.14	-
LAB						
L. delbrueckii MA-9	-	-	-	23.73±0.75	-	-

L. gasseri MA-1	11.51±0.2	-	12.58±0.1	23.18±2.61	-	-
L. gasseri MA-2	-	16.48±0.4	-	24.31±0.63	-	-
L. fermentum MA-8	-	-	-	28.68±1.29	-	-
L. fermentum MA-7	-	-	-	28.42±1.00	-	-

E: Ethanol, M: Methanol, A: Acetone, AM: Ampicillin (10 µg), K: Kanamycin (30 µg), FCA: Fluconazole (25 µg), -: No Activity, LAB: Lactic Acid Bacteria

As with many natural products, the composition of the extracts may change due to differences such as geographical location, plant nutrition and variety, and this may have an effect on the antimicrobial activity. It is also suggested that the gathering areas of plants affect antimicrobial activity due to different soil formations (Pereira et al., 2006; Sousa et al., 2006).

Table 3. MIC, MBC or MFC values of AY and M ethanol olive leaf extracts (µg/µL).

Strains	**AYOL ethanol extract**			**MOL ethanol extract**		
Food and Clinical test microorganisms	MIC	MBC	MFC	MIC	MBC	MFC
E. coli O157:H7	40	40		40	40	
B. subtilis RSKK 244	40	80		40	80	
B. cereus RSKK 863	40	80		40	40	
S. aureus ATCC 25923	20	40		10	10	
P. aeruginosa ATCC 27853	20	40		20	20	
E. coli ATCC 35218	40	80		40	40	
Y. enterolitica ATCC 11175	20	20		40	40	
L. monocytogenes ATCC 7644	40	40		40	80	
S. sonnei MU:57	40	80		40	80	
E. faecalis ATCC 29212	20	40		40	80	
S. enteritidis RSKK 171	20	40		40	80	
C. albicans ATCC 10231	40		80	40		40
C. glabrata RSKK 04019	40		40	40		20
Fish Pathogens						
S. agalactia Pas.Ins. 55118	20	40		40	80	
V. angillarum A4	20	40		40	80	
V. angillarum M1	20	40		40	80	
V. alginolyticus	10	20		40	80	
Y. ruckeri	40	80		40	40	
A. hydrophila ATCC 19570	40	80		40	40	
L. garvieae	40	40		40	80	
LAB						

L. delbrueckii MA-9	40	80	40	80
L. gasseri MA-1	40	20	40	20
L. gasseri MA-2	40	80	40	80
L. fermentum MA-8	20	20	40	40
L. fermentum MA-7	40	20	20	20

MIC: Minimal Inhibition Concentration, MBC: Minimal Bactericidal Concentration, MFC: Minimal Fungicidal Concentration, AYOL: Ayvalık Yaglik olive leaf, MOL: Manzanilla olive leaf, LAB: Lactic Acid Bacteria

Table 4. MIC, MBC and MFC values of AY and M varieties methanol olive leaf extracts (µg/µL).

Strains	**AYOL methanol extract**			**MOL methanol extract**		
Food and Clinical test microorganisms	MIC	MBC	MFC	MIC	MBC	MFC
E. coli O157:H7	40	80		40	80	
B. subtilis RSKK 244	40	40		40	40	
B. cereus RSKK 863	40	40		20	40	
S. aureus ATCC 25923	20	20		20	20	
P. aeruginosa ATCC 27853	20	40		10	10	
E. coli ATCC 35218	40	40		20	20	
Y. enterolitica ATCC 11175	40	40		20	20	
L. monocytogenes ATCC 7644	40	40		20	20	
S. sonnei MU:57	40	80		20	20	
E. faecalis ATCC 29212	40	80		40	40	
S. enteritidis RSKK 171	20	40		40	40	
C. albicans ATCC 10231	20	20		20		20
C. glabrata RSKK 04019	20	20		10		10
Fish Pathogens						
S. agalactia Pas.Ins. 55118	40	80		40	40	
V. angillarum A4	40	80		40	40	
V. angillarum M1	40	40		40	40	
V. alginolyticus	10	10		40	80	
Y. ruckeri	10	20		40	80	
A. hydrophila ATCC 19570	20	20		40	40	
L. garvieae	40	80		40	40	
LAB						
L. delbrueckii MA-9	40	80		40	80	
L. gasseri MA-1	10	10		20	20	
L. gasseri MA-2	40	80		80	80	
L. fermentum MA-8	20	20		10	10	
L. fermentum MA-7	20	20		10	10	

MIC: Minimal Inhibition Concentration, MBC: Minimal Bactericidal Concentration, MFC: Minimal Fungicidal Concentration, AYOL: Ayvalık Yaglik olive leaf, MOL: Manzanilla olive leaf

Table 5: MIC, MBC and MFC values of AY and M varieties acetone olive leaf extracts (μg/μL)

Strains	**AYOL acetone extract**			**MOL acetone extract**		
Food and Clinical test microorganisms	MIC	MBC	MFC	MIC	MBC	MFC
E. coli O157:H7	40	80		40	80	
B. subtilis RSKK 244	40	80		20	20	
B. cereus RSKK 863	40	80		40	80	
S. aureus ATCC 25923	20	40		40	40	
P. aeruginosa ATCC 27853	20	40		40	40	
E. coli ATCC 35218	40	80		40	80	
Y. enterolitica ATCC 11175	40	80		40	80	
L. monocytogenes ATCC 7644	20	20		40	80	
S. sonnei MU:57	40	80		40	40	
E. faecalis ATCC 29212	40	80		40	80	
S. enteritidis RSKK 171	40	40		40	40	
C. albicans ATCC 10231	40		40	40		80
C. glabrata RSKK 04019	40		40	20		20
Fish Pathogens						
S. agalactia Pas.Ins. 55118	40	80		40	80	
V. angillarum A4	40	80		40	80	
V. angillarum M1	40	80		40	80	
V. alginolyticus	20	20		40	80	
Y. ruckeri	40	80		20	20	
A. hydrophila ATCC 19570	40	80		40	80	
L. garvieae	40	80		40	80	
LAB						
L. delbrueckii MA-9	40	80		40	80	
L. gasseri MA-1	40	20		20	20	
L. gasseri MA-2	40	80		40	80	
L. fermentum MA-8	40	20		40	20	
L. fermentum MA-7	40	20		40	20	

MIC: Minimal Inhibition Concentration, MBC: Minimal Bactericidal Concentration, MFC: Minimal Fungicidal Concentration, AYOL: Ayvalık Yaglik olive leaf, MOL: Manzanilla olive leaf

In a report studying the activity of extracts from olive leaves, *B. subtilis* was the least sensitive, followed by *E. coli*, *P. aeruginosa*, *S. pneumoniae* and *S. aureus* (Markin et al., 2003). The similar results can be seen in the current study, *B. cereus* was more sensitive to OLE than *B. subtilis*, but was not the most sensitive microorganism.

The research investigating the effect of olive leaf on fish pathogens has been very limited. In a study, the commercially purchased olive extract was applied to hot-smoked rainbow trout (*Oncorhynchus mykiss*) fillets. According to the results of the microbiological evaluation, the shelf life of the control group was observed as 21 days, while the group that was treated with olive leaf extract exceeded the microbiological limit value on the 42nd day.

Thus, it was determined that olive leaf extract was significantly effective on shelf life (Mutlu & Bilgin, 2016). Korukluoğlu et al. (2010) was extracted olive leaf sample with acetone solvent. In the study, it was determined that the MIC value of the extracts on the test microorganisms as 26-170 μg/ml.

In a study conducted by Asan-Ozusaglam & Gunyaktı (2020), it was determined that *L. gasseri* MA-1, MA-2 and MA-6 strains were sensitive to amikacin, gentamicin, kanamycin, penicillin G antibiotics. *L. gasseri* MA-1, MA-2 and MA-6 strains have gamma hemolytic activity, have bile tolerance and the ability to survive at low pH. The LAB strains have an inhibition zone diameter of 14.29 mm -1.87 mm on the pathogenic microorganisms tested. In their study, they reported that the *L. gasseri* MA-2 strain exhibited promising probiotic properties.

In a study, a sensory evaluation of olive leaf ethanol extracts was performed after 7 days by 15 participants on probiotic *Lactobacillus acidophilus* yogurt containing olive leaf extracts of various concentrations. Significant differences between samples ($p > 0.05$) showed that increased olive leaf extract resulted in favourable taste, color, aroma, and thickness (Marhamatizadeh et al., 2013). Another study investigating the effects of spearmint on bacterial growth showed that increased levels of spearmint promoted the growth of *Lactobacillus acidophilus* and bifidobacteria in probiotic milk and yogurt (Marhamatizadeh et al., 2011). The basic characteristics of consumption of probiotic products are their medicinal properties.

The SPF value of AYOL and MOL extracts were also determined. The obtained results were calculated according to the Mansur equation and the results are given in Table 6. The SPF values of AYOL and MOL extracts were varied from 24.02 to 25.69. When the obtained SPF values were compared with the values given in Imam et al., 2015, the percentage of UV protection of AYOL and MOL extracts was found to be approximately 96%.

UV spectrophotometry is used as a simple, fast, low-cost reagent that can be used for in vitro measurements of SPF values in many cosmetic formulations. In recent years, natural compounds and bioactive products have attracted great interest as UV protectors due to their safe use, environmental problems, and few side effects as well as their antioxidant properties.

The SPF of the commercial cream and AYOL or MOL extracts mixture formulation and the commercial cream were tested (Table 6). Generally, it was observed that the mixture of AYOL and MOL extracts and cream mixture showed a higher SPF value than the commercial cream. AYOL methanol extract and cream mixture had the highest SPF value of 11.26 at 10 mL concentration, and acetone extract had the lowest value was 0.5 at 2.5 ml concentration. The highest value of the commercial cream was 1.29 at 10 ml concentration and the lowest value was 0.16 at 2.5 ml concentration. According to Table 6 (Imam et al., 2015), the highest UV protection percentage was evaluated as approximately 90% for the AYOL methanol extract and the commercial cream mixture.

Table 6. SPF values of AY and M variety olive leaf extracts and commercial cream mixture.

Extracts	AY Extract	M Extract	AY Extract + Cream			M Extract + Cream			Cream		
			2.5 ml	5 ml	10 ml	2.5 ml	5 ml	10 ml	2.5 ml	5 ml	10 ml
E	26.51	25.31	0.26	0.90	7.74	0.32	1.32	11.73	0.16	0.47	1.29
M	24.02	25.40	0.06	1.09	11.26	0.43	0.45	9.89	0.16	0.47	1.29
A	25.09	25.69	0.05	1.06	10.30	0.23	0.64	5.88	0.16	0.47	1.29

E: ethanol, M: methanol, A: acetone; AY: Ayvalık Yaglik; M: Manzanilla

Sunscreen creams can effectively absorb or reflect sunlight, particularly in the UV range (Wilkinson et al., 1982). Some of the ingredients in sunscreens are synthetic substances that can have toxic effects. Natural substances are harmless and believed to be safer to use. Natural ingredients such as olives, aloe vera, tomatoes, pomegranates, green tea, cucumbers and grapes, and botanical ingredients have the potential to block UV rays and thus have potential as sunscreens (Goswami et. al., 2013; Henny & Dachriyanus, 2015). In a study, they observed that olive oil was added to the cream formulation and it was determined that the cream enhanced SPF protection. The best sunscreen formulation for refined tomato extracts, sunscreens have an SPF value of 21.09 and provide excellent protection against UV rays (Sjahjadi & Lucida, 2021).

CONCLUSION

The antimicrobial activity and sunscreen of olive leaf extracts of Ayvalık Yaglik and Manzanilla varieties were investigated. As a result of the research, it was observed that the olive leaf extract has antimicrobial activity against the tested food-borne and clinical and fish pathogen microorganisms. In addition, the results obtained showed that the olive leaf extract was capable of absorbing UV light, thus demonstrating its ability to protect against UV light. It is a better, cheaper, and safer alternative to the harmful chemical sunscreens currently used in the industry. The extracts that do not inhibit the growth of lactic acid bacteria from human milk can be used in fermented products. The fact that the olive plant is an evergreen and its easy production enhances the economic importance of this plant. The research results can be used for the development of functional foods and the preservation of foods. This study creates new alternatives to new health-based food and cosmetics markets to protect human health.

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REFERENCES

Ahmed A.M. Rabii N.S. Garbaj A.M. & Abolghait S.K. 2014. Antibacterial effect of olive (*Olea europaea* L.) leaves extract in raw peeled undeveined shrimp (*Penaeus semisulcatus*). Int. J. Veter. Sci. Med., 2, 53–56.

Asan-Ozusaglam M. & Gunyakti A. 2020. Investigation of *Lactobacillus gasseri* MA-2 as probiotic candidate. Cumhuriyet Science Journal, 41(1), 228-234.

Ayana B. & Turhan K.N. 2009. Use of antimicrobial methylcellulose films to control *Staphylococcus aureus* during storage of Kasar cheese. Packag. Technol. Sci. 22, 461–469.

Bambal V., Wyawahare N., Turaskar A. & Mishra M. 2011. Study of sunscreen activity of herbal cream containing flower extract of *Nyctanthes arbortrıstıs* L. and *Tagetes erecta* L., International Journal of Pharmaceutical Sciences Review and Research, 11(1), 142-146.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuño, A. D. R. J., & Del Rio, J. A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chemistry, 68(4), 457-462.

Ben-Othman S., Jõudu I. & Bhat, R. 2020. Bioactives from agri-food wastes: Present insights and future challenges. Molecules, 25, 510.

Boudhrioua N., Bahloul N., Slimen I.B. & Kechaou, N. 2008. Comparison on the total phenol contents and the color of fresh and infrared dried olive leaves. Ind. Crops Prod. 9, 412–419 https://doi. org/10.1016/j.indcrop.2008.08.001.

Brahmi F., Flamini G., Issaoui M., Dhibi M., Dabbou S., Mastouri M. & Hammami M. 2012. Chemical composition and biological activities of volatile fractions from three Tunisian cultivars of olive leaves. Med. Chem. Res. 21, 2863–2872.

Brown P. & Dawson M.J. 2015. A perspective on the next generation of antibacterial agents derived by manipulation of natural products. In Progress in Medicinal Chemistry; Elsevier B.V.: Oxford, UK, Volume 54, pp. 135–184.

Chen X., Sullivan D.A., Sullivan A.G., Kam W.R. & Liu Y. 2018. Toxicity of cosmetic preservatives on human ocular surface and adnexal cells. Experimental Eye Research, 170, 188–197. https://doi.org/10.1016/J.EXER.2018.02.020.

Erdogan Z.Ö. & Turhan K.N. 2012. Olive leaf extract and usage for development of antimicrobial food packaging. In Science against Microbial Pathogens: Communicating Current Research and Technological Advances; Formatex: Norristown, PA, USA, pp. 1094–1101.

Fuller R. 1989. Probiotics in man and animals. The Journal of Applied Bacteriology, 66(5), 365-378.

Furneri P.M., Marino A., Saija A., Uccella N. & Bisignano G. 2002. In vitro antimycoplasmal activity of oleuropein. Int. J. Antimicrob. Age., 20, 293-296.

Goreishi S.M. & Shahrestani, R. G. 2009. Subcritical water extraction of mannitol from olive leaves. J. Food Eng. 93, 474–481.

Goswami P.K., Samant M. & Srivastava R. 2013. Natural Sunscreen Agents: A Review. Scholars Academic Journal of Pharmacy, 2(6),458-463.

Henny S. & Dachriyanus L. 2015. Formulation of Sunscreen Cream of Germanicol cinnamate from the Leaves of Tabat barito (*Ficus deltoides Jack*) and an Assay of its' Sun Protection Factor. Int. J. Pharm. Sci. Rev. Res. 32(1), 104-107.

Imam S., Azhar I. & Mahmood ZA. 2015. In-vitro evaluation of sun protection factor of a cream formulation prepared from extracts of *Musa accuminata* (L.), *Psidium gujava* (L.) and *Pyrus communis* (L.). Pharmaceutical and Clinical Research 8(3), 234-237.

Jemai H., El Feki, A. & Sayadi S. 2009. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. Journal of Agricultural and Food Chemistry, 57(19), 8798-8804.

Karygianni L., Cecere M., Skaltsounis A.L., Argyropoulou A., Hellwig E., Aligiannis N., Wittmer A. & Al-Ahmad A. 2014. High-level antimicrobial efficacy of representative mediterranean natural plant extracts against oral microorganisms. BioMed Res. Int., 1–8.

Khalil M.M., Ismail E.H., El-Baghdady K.Z. & Mohamed D. 2014. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arab. J. Chem. 7, 1131–1139.

Korukluoğlu M., Şahan Y., Yiğit A., Tümay-Özer E. & Gücer Ş. 2010. Antibacterial activity and chemical constitutions of *Olea europaea* L. leaf extracts. Journal of Food Processing and Preservation, (34), 383-396.

Lafka T.I., Lazou A., Sinanoglou V. & Lazos E. 2013. Phenolic extracts from wild olive leaves and their potential as edible oils antioxidants. Foods, 2, 18–31.

Lee O.H. & Lee B.Y. 2010. Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea leaf* extract. Bioresour. Technol., 101, 3751–3754.

Liu Y., McKeever L.C. & Malik N.S.A. 2017. Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. Front. Microbiol., 2(8), 113.

Mansur JS., Breder MNR., Mansur MCA. & Azulay RD. 1986. "Correlaçäo entre a determinaçäo do fator de proteçäo solar em seres humanos e por espectrofotometria". Anais Brasileiros De Dermatologia, 61(3), 121-124.

Marhamatizadeh M.H., Ehsandoost E., Gholami P. & Mohaghegh M.D. 2013. Effect of Olive leaf extract on growth and viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* for production of probiotic milk and yoghurt. International Journal of Farming and Allied Sciences, 2-17, 572-578.

Marhamatizadeh MH., Karmand M., Farokhi AR., Rafatjoo R. & Rezazade S. 2011. The effects of malt extract on the increasing growth of probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic milk and yoghurt. J. Food Technol. Nutr., 8, 78-84.

Markin D., Duek L. & Berdicevsky I. 2003. In vitro antimicrobial activity of olive leaves. Mycoses, 46, 132–136.

Matsui M. S., Hsia A., Miller J. D., Hanneman K., Scull H., Cooper K. D. & Baron E. 2009. Non-sunscreen photoprotection: antioxidants add value to a sunscreen. Journal of Investigative Dermatology Symposium Proceedings, 14 (1), 56-59.

Menanteau-Ledouble S., Lawrence M.L. & El-Matbouli M. 2018. Invasion and replication of *Yersinia ruckeri* in fish cell cultures. BMC Vet. Res. 14, 1–11.

Mutlu A. & Bilgin Ş. 2016. Zeytin (*Olea europaea* L.) Yaprağı ve Yağ Gülü (*Rosa damascene* Mill.) Ekstraktlarının Buzdolabı Koşullarında (4±1°C) Depolanan Sıcak Dumanlanmış Alabalık (*Oncorhynchus mykiss*) Filetolarının Raf Ömrüne Etkisi. LIMNOFISH-Journal of Limnology and Medicinal Chemistry, 14, 8533-8538.

Nekooeian A. A., Khalili A. & Khosravi M. B. 2014. Oleuropein offers cardioprotection in rats with simultaneous type 2 diabetes and renal hypertension. Indian Journal of Pharmacology, 46(4), 398.

Nichols J. A. & Katiyar S. K. 2010. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. Archives of dermatological research, 302(2), 71-83.

Onuk E., Durmaz Y., Çiftci A. & Pekmezci G. 2015. Çeşitli Balık Türlerinden İzole Edilen Patojen Bakteriler ve Antibiyotik Direnç Profilleri. 10-3.https://doi.org/10.17094/avbd.08517.

Pereira A.P., Ferreira I.C., Marcelino F., Valentão P., Andrade P.B., Seabra R., Estevinho L. & Bento A., Pereira J.A. 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. cv. Cobrançosa) leaves. Molecules, 12, 1153–1162. https ://doi.org/10.3390/12051153.

Pereira JA., Pereira APG., Ferreira ICFR., Valenta OP., Andrade PB., Seabra R., Estevinho L. & Bento A. 2006. Table olives from portugal: phenolic compounds, antioxidant potential and antimicrobial activity. J. Agric. Food Chem. 54, 8425−8431.

Poudyal H., Campbell F. & Brown L. 2010. Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. J. Nutr., 140, 946–953.

Raymond C.R., Paul J.S. & Marian E.Q. 2009. Handbook of Pharmaceutical Excipients (6th ed.). Grayslake, IL: Pharmaceutical Press and American Pharmacist Association.

Riva A., Algaba I. M. & Pepió M. 2006. Action of a finishing product in the improvement of the ultraviolet protection provided by cotton fabrics. Modelisation of the effect. Cellulose, 13(6), 697-704.

Schaafsma G. 2008. Lactose and lactose derivatives as bioactive ingredients in human nutrition. International Dairy Journal, 18(5), 458-465.

Sjahjadi F. R. & Lucida H. 2021. Formula Optimization of a Sunscreen Cream of Tomato‘s Purified Extract. In 2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021), pp. 42-48, Atlantis Press.

Sousa A., Ferreira I.C.F.R., Calhelha R., Andrade P.B., Valentão P., Seabra R., Estevinho L., Bento A. & Pereira J.A. 2006. Phenolics and antimicrobial activity of traditional stoned table olives “alcaparra”. Bioorganic & Medicinal Chemistry, 14(24), 8533- 8538.

Sudjana A.N., D’Orazio C., Ryan V., Rasool N.; Ng J., Islam N., Riley T.V. & Hammer, K.A. 2009. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. Int. J. Antimicrob. Agents, 33, 461–463.

Suez O. 2019. Bacteria associated with fresh-water aquaculture tilapia fish (*Oreochromis niloticus*) in Suez, Egypt. Food Sci. Nutr. Res. 2, 1–7.

Vogel P., Machado I. K., Garavaglia J., Zani V. T., de Souza D. & Dal Bosco S. M. 2015. Polyphenols benefits of olive leaf (*Olea europaea* L) to human health. Nutrición Hospitalaria, 31(3), 1427-1433.

Wilkinson JB. & Moore R. 1982. Harry’s Cosmeticology: The Principles and Practice and Practice of Modern Cosmetic. Seventh Ed. London: Leonard Hill Book.