J Skin Stem Cell. 2023 June; 10(2):e137580. https://doi.org/10.5812/jssc-137580.

Published online 2023 July 7. **Research Article**

A Natural Additive Alternative to the Cosmetics Industry: Hazelnut and Its Waste Products

Ali Saglam [1] and Meltem Asan-Ozusaglam [1, *]

[1]Department of Molecular Biology and Genetic, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey

[*]*Corresponding author*: Department of Molecular Biology and Genetic, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey. Email: meltemozusaglam@gmail.com

Received 2023 May 13; **Revised** 2023 June 12; **Accepted** 2023 June 14.

Abstract

Background: Today, individuals tend to use natural products instead of synthetic additives in many areas. The hazelnut tree produces many by-products and fruit. Nuts and their by-products are rich in bioactive compounds.

Objectives: This study investigated the biological activities of water and ethanol extracts obtained from hazelnut and its waste products to determine their potential usage in the cosmetic industry.

Methods: In this experimental study, disc diffusion test, minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), or minimum fungicidal concentration (MFC) were applied to evaluate the antimicrobial potential of the extracts. The sun protection factor (SPF) of the extracts and commercial cream + extract mixtures was determined in vitro. In addition, antimicrobial cream formulations containing hazelnut extracts and *Limosilactobacillus fermentum* MA-7 probiotic candidate lactic acid bacteria from human milk were developed for the pharmaceutical industry to prevent infections. The good diffusion test was applied against test microorganisms to evaluate antimicrobial activity.

Results: The hazelnut husk methanol extract had the highest inhibition zone diameter (19.41 mm) against *Yersinia ruckeri*. The MIC, MBC, or MFC of the extracts ranged from 1.25 to > 40 $\mu g/\mu L$. The SPF values of the extracts (range: 6.85-27.64) and commercial cream + extract (range: 11.92-26.28) mixtures were determined in vitro to obtain their potential use in sunscreens. The cream groups containing hazelnut extracts and probiotics showed a high antimicrobial effect on the tested microorganisms. The results of statistical analysis indicated that the cream + extract + probiotic pellet + probiotic supernatant group was statistically significant ($P < 0.05$) compared to other test groups.

Conclusions: The results showed that hazelnuts and their by-products have the potential to be used as a natural source of antimicrobials. Hazelnut and its by-products can be an alternative to synthetic antimicrobials and sunscreens in the cosmetic industry as a natural bioactive substance. In addition, it might contribute to the country's economy by evaluating hazelnuts and their waste and by-products resulting from their processing in the cosmetic industry.

Keywords: Antimicrobial, Cream Formulation, Extract, Probiotic, Solar Protection

1. Background

Hazelnut (*Corylus avellana L.*), of the Betulaceae family, is one of the nuts containing high amounts of nutrients and lipids (1). The Ministry of Agriculture and Forestry of the Republic of Türkiye has published Turkey as the country producing the most hazelnuts worldwide, with 665 thousand tons in 2020 (2). Turkey, which meets approximately 65-70% of the world's hazelnut production, is the most important hazelnut producer in the world (3). Hazelnut, which has a rich phenolic content, is used directly as a snack in human nutrition and as an ingredient in various food products (4).

The fact that it has a rich content in terms of functional food composition will increase the interest in hazelnut in Iran and the world. It is known that some waste products (e.g., green leafy cover and shell) are formed during hazelnut production. The evaluation and functionalization of waste and by-products produced as a result of production will prevent environmental pollution and increase the economy and interest in hazelnut (1). Therefore, the importance of worldwide hazelnut production can be increased by developing new alternatives for the use of hazelnuts and their wastes as

bioactive substances in the cosmetic industry.

Probiotics are defined as live microorganisms that provide various benefits to the host (5). Probiotic microorganisms mainly have beneficial effects, such as strengthening the epithelial barrier, stabilizing the gastrointestinal microbiota, degrading toxins, producing antimicrobials, and enhancing immune response (6). *Limosilactobacillus fermentum*, one of the probiotic microorganisms, shows antioxidant, antimicrobial, and anti-photoaging properties and intestinal barrier function (7-9). Under the Generally Recognized as Safe status, *L. fermentum* can be used as a safe food, drug, and cosmetic and is not subject to pre-market approval requirements (10). It also has healing properties on skin health and skin flora (11).

In recent years, microorganisms have become resistant to antibiotics, and the inadequacy of synthetic drugs has increased the interest in natural-origin drugs (12, 13). Scientists have proven the medicinal effect of plant-based products on microorganisms (14, 15). Food poisoning caused by pathogenic microorganisms in food poses an important health problem worldwide. Pathogenic microorganisms cause food spoilage, digestive system diseases, or nervous system disorders (16).

Nosocomial infections are one of the serious health problems caused by clinically pathogenic microorganisms despite sterilization and disinfection precautions (17). Bacterial infections of the skin and subcutaneous tissue are among the most common nosocomial infections in the community (18). Skin infections caused by yeasts, especially those associated with *Candida albicans*, are quite common. In addition, *Candida* species are emerging with new strains with greater resistance to existing antifungal agents (19). Diseases caused by pathogenic bacteria have also increased in aquaculture (20). Therefore, this issue causes significant economic loss in aquaculture (21). Plants can produce compounds with many biological activities effective against pathogens. Consequently, plant extracts are expected to be effective in drug-resistant pathogenic microorganisms (22).

Different types of electromagnetic radiation come from the sun to the earth. Ultraviolet (UV) radiation is a form of "invisible and non-ionizing" radiation to which humans are exposed throughout their lives (23). The UV radiation, with wavelength energy of 100-400 nm, spans between the X-ray and visible light spectrum. These rays are UV-A, UV-B, and UV-C. The ozone layer absorbs 100% of UV-C and approximately 90% of UV-B and almost does not absorb UV-A (24). Exposure to UV radiation produces harmful effects on the skin, such as dryness, wrinkles, skin cancer, and pigment abnormalities (25). Using sunscreen products can prevent the skin from the harmful effects of UV rays and negative skin effects (26). The bioactive substances of plants can help prevent harmful rays from the sun and protect the skin.

2. Objectives

The present study aimed to determine the potential usage of hazelnut and its by-products as natural bioactive additives in the cosmetic industry. Therefore, the antimicrobial effect of various extracts obtained from hazelnut and its waste products on foodborne, clinical, and fish-originated microorganisms was investigated. Additionally, the sun protection factor (SPF) value of the extracts and the commercial cream + extract mixtures developed by using hazelnut extracts was assayed to reveal the potential use as cheaper and safer alternatives to sunscreens containing harmful chemicals used in cosmetics. Moreover, cream formulations containing hazelnut extracts and *L. fermentum* probiotic microorganisms were developed, and their antibacterial and antifungal activities on *Escherichia coli* O157:H7 and *C. albicans* ATCC 10231 were investigated for the cosmetic industry.

3. Methods

3.1. Preparation of Hazelnut Extracts

Hazelnut whole fruit (with brown shell), green husk (green leafy shell), and leaf samples were obtained from Ordu/Turkey in July 2021. The hazelnut materials were separately air-dried at room temperature. In the extraction, 10 gr of powder obtained from whole hazelnut fruit (WHF), hazelnut green husk (HH), and hazelnut leaves (HL) was separately extracted with 30 mL of water or methanol using a hot water bath for 24 hours. The solvents were evaporated after extraction. The extracts were dissolved with dimethyl sulfoxide (DMSO) and then sterilized with 0.45 μm Millipore filters. They were then kept in dry conditions at 4°C until use.

3.2. Test Microorganisms

The antimicrobial activity of water and methanol extracts from WHF, HH, and HL was tested against 10 pathogenic microorganisms. The foodborne or clinical (i.e., *E. coli* O157:H7, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 171, *Candida glabrata* RSKK 04019, and *C. albicans* ATCC 10231) and fish pathogens

(*Aeromonas hydrophila* ATCC 19570, *Yersinia ruckeri*, and *Lactococcus garvieae*) were used as test microorganisms. Foodborne or clinical pathogens were cultured at 37°C in tryptic soy broth (TSB) or nutrient broth (NB) media for 24 hours. The yeasts were grown at 30°C in yeast peptone dextrose media for 48 hours. The *A. hydrophila* ATCC 19570 fish pathogen was cultured at 25°C in NB. *Y. ruckeri* and *L. garvieae* fish pathogens were grown in TSB medium at 25°C for 24 hours.

3.3. Disc Diffusion Test

The antimicrobial activity of the hazelnut extracts against the test microorganisms was determined using the disc diffusion test (27). The test microorganisms were adjusted to 0.5 McFarland concentrations. Then, the microbial suspensions (100 μL) were spread on the solid medium. The sterile filter discs (6 mm in diameter) were placed on the solid medium. The hazelnut extracts (20 μL, 2000 μg/disc) were dropped onto the discs. The plates were then incubated at 37°C for 24 hours for bacterial strains and at 30°C for 48 hours for yeast. Ampicillin (10 μg/disc), kanamycin (30 μg/disc), and fluconazole (25 μg/disc) were used as positive controls. The extract solvent DMSO was used as the negative control. The inhibition zone diameters were measured using Vernier calipers. The study was repeated twice.

3.4. Microdilution Assay

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) values of the extracts were obtained by the microdilution method against test microorganisms (27). The medium and hazelnut extracts were added to the tubes and diluted to determine the MIC of the extracts. The microbial suspension (0.5 McFarland) was added into the tubes containing various extract concentrations (40, 20, 10, 5, 2.5, 1.25, and 0.625 μg/μL). The mixture of medium and test microorganisms (without extract) was used as a positive control in the study. After mixing, the tubes were incubated for 24 and 48 hours at appropriate temperatures. The lowest hazelnut extract concentration that inhibited the growth of test microorganisms was recorded as the MIC value. The MBC or MFC values were obtained by inoculating a 5 μL sample from the incubated tubes on solid media. After the incubation, the lowest concentration without microbial growth was recorded as the MBC value, and the lowest concentration without fungal growth was recorded as the MFC value. The data are expressed in μg/μL.

3.5. Determination of In-vitro Sun Protection Factor of Extract

The SPF values of hazelnut extracts are based on the in vitro determination of extracts (2 μg/μL) diluted in ethanol (96%). The diluted solutions were measured by a spectrophotometer (Beckman Coulter Inc., United States) in a wavelength range of 290 - 320 nm (UV-B) at 5 nm intervals. The SPF value was calculated using the Mansur equation (28) given in the subsequent section 1.

In addition, the SPF values of the commercially available cream and hazelnut extract mixture were also examined. For this purpose, Imam et al.'s (29) and Bambal et al.'s (30) studies were used after modification. Commercial cream (10% w/v) and the extract (5% w/v) were mixed and made up to the final volume with distilled water. The mixture was diluted with ethanol (40%) and then sonicated for 5 minutes. The mixtures with a final volume of 2.5 - 5 - 10 mL were measured in the wavelength range of 290 - 320 nm (UV-B), as mentioned above. The SPF values of the cream and extract mixtures were calculated using the Mansur equation (28) as follows:

$$SPF = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda)$$

CF: Correction factor (10); EE (λ): Erythemetogenic effect radiation wavelength (λ); I (λ): Intensity of sunlight at the wavelength (λ); Abs (λ): Absorbance value of extracts at the wavelength (λ)

3.6. Antimicrobial Activity of Cream Formulations

The antimicrobial activity of the cream formulations was determined with a developed method using Handali et al.'s (31) and Chen et al.'s (32) studies. The antimicrobial cream formulations contain commercial cream and/or hazelnut extracts and/or probiotic candidate strains originating from human milk *Limosilactobacillus fermentum* MA-7. The *L. fermentum* MA-7 strain has good probiotic properties (33). The precipitated pellet and supernatant obtained by the centrifugation of the active culture of *L. fermentum* MA-7 strain were used in different cream formulations. In this study, 10% cream, 20% hazelnut extracts, and 10% *L. fermentum* MA-7 pellets were used in the content of the prepared cream groups. In addition, the groups containing the supernatant of the *L. fermentum* MA-7 strain were filled with a supernatant to the final volume, and the cream groups that did not contain the *L. fermentum* MA-7 strain were completed with distilled water. The extract concentration of all groups containing extract was 200 μg/μL. Table 1 shows the prepared cream groups. The cream groups were homogenized and sonicated for 30 minutes. The cream

formulations were then sterilized using a 0.45 μm filter and kept at 4°C in dry conditions until use.

Table 1. Cream Formulations Containing Hazelnut Extract and Probiotic

	Cream Groups Containing Hazelnut Extract and Probiotic
C	Cream
CE	Cream + extract
CP	Cream + probiotic pellet
CS	Cream + probiotic supernatant
CEP	Cream + extract + probiotic pellet
CES	Cream + extract + probiotic supernatant
CPS	Cream + probiotic pellet + probiotic supernatant
CEPS	Cream + extract + probiotic pellet + probiotic supernatant

The antimicrobial activity of the antimicrobial cream groups was tested against *E. coli* O157:H7 and *C. albicans* ATCC 10231 using the good diffusion method. The test microorganisms were adjusted to a 0.5 McFarland concentration after washing twice with saline. Then, the microbial suspensions (100 μL) were spread onto the solid medium, and 100 μL of cream groups was dropped into the wells (a diameter of 6 mm). The plates were incubated at appropriate conditions, as mentioned before. After the incubation, the inhibition zones were measured with Vernier calipers. The study was conducted in triplicate.

3.7. Statistical Analysis

The antimicrobial activity of the cream formulations was analyzed using GNU SPSS software (version 25.0), and statistical significance was confirmed by one-way analysis of variance with Tukey's post-hoc test. The difference between the cream groups was considered statistically significant at $P < 0.05$.

4. Results

4.1. Antimicrobial Activity of Extracts

In the present study, the antimicrobial effects of various extracts from hazelnut and its by-products on foodborne, clinical, and fish-originated microorganisms were determined using disc diffusion and microdilution assays. The hazelnut extracts showed antimicrobial activity against most of the tested microorganisms (Table 2). The hazelnut leaf methanol (HLM) extract had the highest inhibition effect against *P. aeruginosa* ATCC 27853, with an inhibition zone diameter of 15.55 mm. The hazelnut husk methanol (HHM) extract showed the greatest inhibition effect against *C. glabrata* RSKK 04019 (17.25 mm) among fungal test microorganisms. *Yersinia ruckeri* is the most inhibited fish pathogen by the HHM extract, with an inhibition zone diameter of 19.41 mm. The MIC, MBC, or MFC values of hazelnut extracts varied from 1.25 to > 40 μg/μL (Table 3). The lowest MIC value (5 μg/μL) was determined against *P. aeruginosa* ATCC 27853 for the hazelnut leaf water (HLW) extract and against *L. garvieae* for the hazelnut husk water (HHW) extract. The best MBC or MFC value (1.25 μg/μL) was obtained against *A. hydrophila* ATCC 19570 for the HHM extract.

4.2. In-vitro Sun Protection Factor of Extracts

The SPF values of WHF, HH, and HL extracts were determined in vitro spectrophotometrically. The SPF values of the hazelnut extracts varied from 6.85 to 27.64 (Table 4). As a result of spectrophotometric measurements, the methanol hazelnut extracts generally had higher SPF values than the water extracts. The highest SPF value was 27.64 for the HLM extract. The methanol hazelnut extracts presented over 96% UV blocking, according to Imam et al.'s study (29).

After determining the SPF values of the extracts, they were mixed with the commercial cream, and then the SPF values of the cream mixture were obtained. The results are shown in Table 5. The SPF value of the commercial cream was determined as the control. The SPF values of the cream-extract mixtures showed that the highest SPF values were observed for the HLM extract as 2.02, 9.84, and 26.28 at concentrations of 2.5, 5, and 10 mL, respectively.

4.3. Antimicrobial Activity of Cream Formulations

The cream formulations prepared with hazelnut extracts and probiotics showed antimicrobial activities against *E. coli* O157:H7 and *C. albicans* ATCC 10231 (Table 6). The Cream Extract (CE), Cream Probiotic Supernatant (CS), Cream Extract Probiotic Pellet (CEP), Cream Extract Probiotic Supernatant (CES), Cream Probiotic Pellet Probiotic Supernatant (CPS), and Cream Extract Probiotic Pellet Probiotic Supernatant (CEPS) groups showed statistically significant antimicrobial activity, compared to the C and CP groups, against *E. coli* O157:H7 ($P < 0.05$). The mixture of the HLM extract containing CEPS showed the highest activity on the *E. coli* O157:H7 with an inhibition zone diameter of 22.61 mm. The antimicrobial activity results of the CE group prepared with the HHM or HLM extract on *C. albicans* ATCC 10231 indicated a statistically significant difference ($P < 0.05$), compared to the control, except for other CE test groups. In addition, CEP groups on *C. albicans* ATCC 10231, except for the HHW extract, showed statistically significant antimicrobial activity (P

Table 2. Disc Diffusion Assay Results of Hazelnut Extracts

	Inhibition Zone Diameter (mm ± SD)							
	WHFW	**HHW**	**HLW**	**WHFM**	**HHM**	**HLM**	**AM**	**K**
Foodborne and clinical pathogens								
Escherichia coli O157:H7	10.27 ± 0.50	8.3 ± 0.00	NA	13.51 ± 0.20	12.24 ± 0.09	8.14 ± 0.49	16.81 ± 0.20	12.97 ± 0.30
Enterococcus faecalis ATCC 29212	6.08 ± 0.02	7.44 ± 1.96	NA	8.72 ± 1.17	10.60 ± 0.07	8.28 ± 0.65	30.27 ± 0.90	19.36 ± 0.10
Listeria monocytogenes ATCC 7644	6.09 ± 0.11	6.10 ± 0.07	NA	NA	8.58 ± 1.43	8.14 ± 0.49	17.76 ± 0.00	19.33 ± 0.40
Pseudomonas aeruginosa ATCC 27853	11.47 ± 0.99	9.23 ± 0.28	10.55 ± 0.77	14.83 ± 0.40	14.60 ± 0.36	15.55 ± 1.31	21.04 ± 0.80	19.91 ± 0.50
Salmonella enteritidis RSKK 171	10.65 ± 1.53	8.20 ± 1.12	6.03 ± 0.00	13.59 ± 0.37	12.24 ± 0.83	8.14 ± 0.49	14.02 ± 0.30	15.48 ± 1.40
Fungal pathogens							**FCA**	
Candida glabrata RSKK 04019	12.62 ± 0.18	12.46 ± 1.11	9.79 ± 0.16	16.67 ± 1.50	17.25 ± 0.99	10.46 ± 0.10	20.35 ± 0.10	
Candida albicans ATCC 10231	9.47 ± 0.37	8.12 ± 1.80	6.66 ± 0.71	11.54 ± 0.91	11.7 ± 0.02	NA	NA	
Fish pathogens							**AM**	**K**
Aeromonas hydrophila ATCC 19570	11.08 ± 0.41	9.94 ± 0.46	NA	18.93 ± 0.57	19.35 ± 0.67	10.59 ± 1.67	12.2 ± 0.06	16.16 ± 0.05
Yersinia ruckeri	10.93 ± 1.28	9.24 ± 0.45	7.49 ± 0.94	15.72 ± 2.29	19.41 ± 0.08	7.49 ± 0.40	32.3 ± 0.15	17.54 ± 0.03
Lactococcus garvieae	12.01 ± 1.84	9.16 ± 1.15	NA	13.91 ± 0.68	18.98 ± 0.41	11.0 ± 0.43	33.1 ± 0.12	23.05 ± 0.14

Abbreviations: SD, standard deviation; WHFW, whole hazelnut fruit water; HHW, hazelnut husk water; HLW, hazelnut leaf water; WHFM, whole hazelnut fruit methanol; HHM, hazelnut husk methanol; HLM, hazelnut leaf methanol; AM (10 μg), ampicillin; K (30 μg), kanamycin; FCA (25 μg), fluconazole; NA, no activity

Table 3. Minimal Inhibitory Concentration and Minimal Bactericidal Concentration or Minimum Fungicidal Concentration Values of Hazelnut Extracts (μg/μL)

	WHFW		**HHW**		**HLW**		**WHFM**		**HHM**		**HLM**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Foodborne and clinical test pathogens												
Escherichia coli O157:H7	40	40	20	40	20	20	20	20	20	20	20	20
*Enterococcus faecalis*ATCC 29212	10	40	20	20	20	40	20	20	20	20	40	40
Listeria monocytogenes ATCC 7644	10	40	10	40	20	20	40	40	20	20	40	40
Pseudomonas aeruginosa ATCC 27853	10	20	10	> 40	5	10	10	10	5	5	5	5
*Salmonella enteritidis*RSKK 171	10	20	20	40	10	20	20	20	10	10	10	10
Fungal pathogens	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Candida glabrata RSKK 04019	10	10	20	20	10	10	20	20	20	20	20	20
Candida albicans ATCC 10231	20	40	40	> 40	20	20	40	40	40	40	20	20
Fish pathogens	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Aeromonas hydrophila ATCC 19570	10	10	20	20	5	5	10	10	1.25	1.25	1.25	1.25
Yersinia ruckeri	20	40	10	20	20	20	20	20	10	10	20	20
Lactococcus garvieae	20	40	5	20	20	20	20	20	5	5	20	20

Abbreviations: MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; MFC, minimum fungicidal concentration; WHFW, whole hazelnut fruit water; HHW, hazelnut husk water; HLW, hazelnut leaf water; WHFM, whole hazelnut fruit methanol; HHM, hazelnut husk methanol; HLM, hazelnut leaf methanol.

< 0.05) compared to the control group. The CEPS group containing the WHFM extract had the best inhibition zone diameter of 20.94 mm against *C. albicans* ATCC 10231. As a result, the antimicrobial activity of the CEPS group against *E. coli* O157:H7 and *C. albicans* ATCC 10231 was observed as statistically significant ($P < 0.05$) compared to other test groups.

5. Discussion

The indiscriminate, irregular, and excessive use of antibiotics has caused antimicrobial resistance and rendered many drugs ineffective (34, 35). For this reason, there is increasing interest in the development of alternative antimicrobial agents with natural ingredients

Table 4. Sun Protection Factor Values of Hazelnut Extracts

Extracts	SPF Value
WHFW	9.78
HHW	6.85
HLW	9.52
WHFM	25.97
HHM	26.34
HLM	27.64

Abbreviations: WHFW, whole hazelnut fruit water; HHW, hazelnut husk water; HLW, hazelnut leaf water; WHFM, whole hazelnut fruit methanol; HHM, hazelnut husk methanol; HLM, hazelnut leaf methanol; SPF, sun protection factor.

Table 5. Sun Protection Factor Values of Hazelnut Extracts and Commercial Cream Mixtures

Extracts	SPF Value		
	2.5 mL	5 mL	10 mL
Cream (control)	0.16	0.47	1.29
WHFW + cream	0.30	1.53	14.97
HHW + cream	0.22	1.35	11.92
HLW + cream	0.35	2.19	19.89
WHFM + cream	0.38	1.93	17.56
HHM + cream	0.50	2.62	20.63
HLM + cream	2.02	9.84	26.28

Abbreviations: WHFW, whole hazelnut fruit water; HHW, hazelnut husk water; HLW, hazelnut leaf water; WHFM, whole hazelnut fruit methanol; HHM, hazelnut husk methanol; HLM, hazelnut leaf methanol; SPF, sun protection factor.

that can reduce the use of antibiotics. Plants are an almost unlimited source of bioactive compounds that have the potential to be used as antimicrobial agents (36). The antimicrobial effect of herbal extracts is being investigated for new treatments.

In a study evaluating the antimicrobial activity of the water extract from the fruit of three different hazelnut cultivars, the extracts showed high antibacterial activity on gram-positive pathogenic microorganisms (i.e., *Bacillus subtilis*, *B. cereus*, and *Staphylococcus aureus*) with an inhibition zone diameter of 4 to > 9 mm. It was determined that the hazelnut extracts had good antimicrobial activity against the test bacteria with a MIC value of 0.1 mg/mL. In the aforementioned study, it was also determined that the tested gram-negative bacteria and fungi (i.e., *P. aeruginosa*, *Klebsiella pneumoniae, E. coli, C. albicans,* and *Cryptococcus neoformans*) were resistant to the extracts (37).

Özaslan et al. (38) tested the antimicrobial activity of hazelnut husk (green leafy shell) and hazelnut leaf extracts extracted with methanol on test bacteria (i.e., *Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, L. monocytogenes, E. faecalis*, and *E. coli*). The extracts showed an antimicrobial effect against some of the tested bacteria but no inhibitory activity against *E. coli* and *P. vulgaris*. In the current study, all the hazelnut extracts showed antibacterial activity on *E. coli* O157:H7 except for the HLW extract. The results of the current study indicated that hazelnut and their waste products with high antimicrobial activity might be natural antimicrobial alternatives to chemicals.

Sunscreens are products developed to protect against the effects of sunburn, skin cancer, aging, loss of collagen, and undesired skin pigmentation caused by excessive solar UV radiation. Ideal sunscreens should not show allergic reactions or toxic effects; however, the side effects that might occur in their use also bring about various concerns for users (39). In addition, the accumulation of sunscreens in the environment also poses important risks (40). For this reason, the development of sunscreens containing natural substances might be very beneficial for the protection of both users and the environment (41). Ivanovic et al. (42) obtained extracts from the roasted shells of Serbian hazelnuts using various ethanol ratios (10%, 50%, and 96%). Their SPF values were examined spectrophotometrically at 220 and 440 nm intervals. The SPF values of the extracts ranged from 0.24 to 0.31. The extracts were also mixed with benzophenone-3, which is considered a chemical sunscreen substance. The SPF values of the hazelnut extract mixtures were obtained within the range of 4.66 - 4.94. They indicated that hazelnut extracts are not suitable for use as UV-enhancers in cosmetic products. However, in the presented study, the hazelnut extracts showed good solar protection activity. Especially the HLM extract with high SPF values indicated that the extract could be used as a natural UV-booster cosmetic.

Lactic acid bacteria (LAB), which constitute a large group of probiotics, have therapeutic effects, such as improving the immune system, reducing the harmful microorganism population, and providing and maintaining the microorganism balance in the intestinal tract (43). Although antimicrobial activity was not detected against *E. coli* O157:H7 in the HLW extract (100 mg/mL) in the disc diffusion test, a higher concentration of the extract (200 mg/mL) used to determine the antimicrobial activity of cream formulations might have created an inhibition zone. The HLM and WHFM extracts, together with the probiotic candidate LAB strain *L. fermentum* MA-7, might contribute to the cosmetic industry as alternative natural antimicrobial sources to chemical

Table 6. Antimicrobial Activity of Hazelnut Extracts and Probiotic Containing Cream Formulations Against *Escherichia coli* O157:H7 and *Candida albicans* ATCC 10231 [a]

Microorganisms	Inhibition Zone Diameter (mm)								F (P-Value)
	C	CE	CP	CS	CEP	CES	CPS	CEPS	
***Escherichia coli* O157:H7**									
WHFW	NA^{A}	3.88 ± 0.69^{B}	NA^{A}	4.92 ± 1.01^{C}	4.32 ± 0.62^{D}	6.16 ± 0.80^{E}	9.91 ± 0.44^{F}	11.96 ± 0.95^{G}	117.641 (0.000)
HHW	NA^{A}	4.12 ± 0.85^{B}	NA^{A}	4.92 ± 1.01^{C}	3.90 ± 1.43^{D}	4.96 ± 0.45^{E}	9.91 ± 0.44^{F}	12.55 ± 1.37^{G}	76.634 (0.000)
HLW	NA^{A}	3.23 ± 0.701^{B}	NA^{A}	4.92 ± 1.01^{C}	4.56 ± 0.58^{D}	14.56 ± 0.88^{E}	9.91 ± 0.44^{F}	18.32 ± 0.51^{G}	348.665 (0.000)
WHFM	NA^{A}	3.34 ± 0.58^{B}	NA^{A}	4.92 ± 1.01^{C}	6.20 ± 0.11^{D}	18.22 ± 2.06^{E}	9.91 ± 0.44^{F}	22.55 ± 0.75^{G}	257.358 (0.000)
HHM	NA^{A}	2.80 ± 0.68^{B}	NA^{A}	4.92 ± 1.01^{C}	4.02 ± 0.30^{D}	4.34 ± 0.43^{E}	9.91 ± 0.44^{F}	19.41 ± 1.35^{G}	255.449 (0.000)
HLM	NA^{A}	10.09 ± 1.00^{B}	NA^{A}	4.92 ± 1.01^{C}	10.11 ± 0.34^{D}	19.09 ± 0.66^{E}	9.91 ± 0.44^{F}	22.61 ± 0.90^{G}	443.196 (0.000)
***Candida albicans* ATCC 10231**									
WHFW	NA^{A}	NA^{A}	NA^{A}	5.44 ± 0.29^{B}	7.74 ± 0.50^{C}	8.12 ± 0.52^{D}	5.64 ± 0.64^{E}	12.89 ± 0.37^{F}	457.186 (0.000)
HHW	NA^{A}	NA^{A}	NA^{A}	5.44 ± 0.29^{B}	1.32 ± 0.29^{A}	7.85 ± 0.09^{C}	5.64 ± 0.64^{D}	9.28 ± 1.12^{E}	189.087 (0.000)
HLW	NA^{A}	NA^{A}	NA^{A}	5.44 ± 0.29^{B}	6.04 ± 0.37^{C}	7.71 ± 0.58^{D}	5.64 ± 0.64^{E}	12.72 ± 0.87^{F}	282.329 (0.000)
WHFM	NA^{A}	NA^{A}	NA^{A}	5.44 ± 0.29^{B}	5.31 ± 0.37^{C}	10.90 ± 1.17^{D}	5.64 ± 0.64^{E}	20.94 ± 1.58^{F}	260.378 (0.000)
HHM	NA^{A}	5.12 ± 0.53^{B}	NA^{A}	5.44 ± 0.29^{C}	10.24 ± 0.43^{D}	6.87 ± 0.73^{E}	5.64 ± 0.64^{F}	13.52 ± 1.00^{G}	202.585 (0.000)
HLM	NA^{A}	5.96 ± 1.01^{B}	NA^{A}	5.44 ± 0.29^{C}	8.90 ± 0.63^{D}	12.68 ± 0.64^{E}	5.64 ± 0.64^{F}	15.27 ± 0.73^{G}	246.447 (0.000)

Abbreviations: WHFW, whole hazelnut fruit water; HHW, hazelnut husk water; HLW, hazelnut leaf water; WHFM, whole hazelnut fruit methanol; HHM, hazelnut husk methanol; HLM, hazelnut leaf methanol; NA, no activity; C, cream; CE, cream + extract; CP, cream + probiotic pellet; CS, cream + probiotic supernatant; CEP, cream + extract + probiotic pellet; CES, cream + extract + probiotic supernatant; CPS, cream + cream + probiotic pellet + probiotic supernatant; CEPS, cream + extract + probiotic pellet + probiotic supernatant.

[a] Values with the different superscript letters in the lines are significantly different using a one-way analysis of variance followed by Tukey's post-hoc test ($P < 0.05$).

antimicrobials. Montella et al. (44) also stated that hazelnut shell extracts, as a growth-medium component, significantly improved the growth of probiotic strains *Lactobacillus crispatus* P17631 and *L. plantarum* P17630.

5.1. Conclusions

The present study investigated the biological activity of the hazelnut and its by-products to obtain their potential use in the cosmetic industry. The hazelnut extracts showed good antimicrobial activity on all tested microorganisms. The extracts and the cream + extract mixtures with high SPF values can be bioactive-natural protectors as an alternative to synthetic sunscreens. Moreover, the cream formulations prepared with cream, hazelnut extract, and probiotic candidate *L. fermentum* MA-7 showed a high antimicrobial effect on the test microorganisms. Finally, the current study showed that hazelnut and its by-products can be used as a natural substance in the cosmetic industry. As a result, environmental pollution can be prevented, and contribution to the national economy can be provided by the use of waste and by-products resulting from hazelnut processing in various industries.

Footnotes

Authors' Contribution: Study concept and design: A. S. and M. A.; Analysis and interpretation of the data: A. S. and M. A.; Drafting of the manuscript: A. S. and M. A.; Statistical analysis: M. A.

Conflict of Interests: The authors have no conflict of interest to declare.

Funding/Support: This research article was not involved in any project and received no support.

References

1. Vlahova V. The role of hazelnuts (Corylus avellana L.) for the sustainability of the agroecosystem. *New knowl J Sci*. 2020;**9**(4):77–84.
2. *Agricultural products markets*. Republic of Türkiye Ministry of Agriculture and Forestry; 2022, [cited 2023 March 02]. Available from: https://arastirma.tarimorman.gov.tr/tepge/Belgeler/PDF%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Piyasalar%C4%B1/2022-Temmuz%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Raporu/14-FINDIK%20T%C3%9CP%20TEMMUZ%202022.pdf.
3. *Hazelnut Production*. Food and Agriculture Organization of the United Nations (FAO); 2023, [cited 2023 March 02]. Available from: https://www.fao.org/3/x4484e/x4484e03.htm.
4. Spagnuolo L, Della Posta S, Fanali C, Dugo L, De Gara L. Antioxidant and Antiglycation Effects of Polyphenol Compounds Extracted from Hazelnut Skin on Advanced Glycation End-Products (AGEs) Formation. *Antioxidants (Basel)*. 2021;**10**(3). [PubMed ID: 33802107]. [PubMed Central ID: PMC7999557]. https://doi.org/10.3390/antiox10030424.
5. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;**11**(8):506–14. [PubMed ID: 24912386]. https://doi.org/10.1038/nrgastro.2014.66.
6. Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S, Gomez-Llorente C, Gil A. Probiotic mechanisms of action. *Ann Nutr Metab*. 2012;**61**(2):160–74. [PubMed ID: 23037511]. https://doi.org/10.1159/000342079.

7. Zhang DI, Li C, Shi R, Zhao F, Yang Z. Lactobacillus fermentum JX306 Restrain D-galactose-induced Oxidative Stress of Mice through its Antioxidant Activity. *Pol J Microbiol*. 2020;**69**(2):205–15. [PubMed ID: 32548989]. [PubMed Central ID: PMC7324864]. https://doi.org/10.33073/pjm-2020-024.
8. Zhou X, Du HH, Ni L, Ran J, Hu J, Yu J, et al. Nicotinamide Mononucleotide Combined With Lactobacillus fermentum TKSN041 Reduces the Photoaging Damage in Murine Skin by Activating AMPK Signaling Pathway. *Front Pharmacol*. 2021;**12**:643089. [PubMed ID: 33841160]. [PubMed Central ID: PMC8027253]. https://doi.org/10.3389/fphar.2021.643089.
9. Palani Kumar MK, Halami PM, Serva Peddha M. Effect of Lactobacillus fermentum MCC2760-Based Probiotic Curd on Hypercholesterolemic C57BL6 Mice. *ACS Omega*. 2021;**6**(11):7701–10. [PubMed ID: 33778280]. [PubMed Central ID: PMC7992159]. https://doi.org/10.1021/acsomega.1c00045.
10. GRAS. *Evaluation of The Generally Recognized As Safe (Gras) status of Lactobacillus fermentum LfQi6 as a food ingredient*. GRAS Notice (GRN) No. 988; 2020.
11. Lee JY, Kim Y, Kim JI, Lee H, Moon G, Kang C. Improvements in Human Keratinocytes and Antimicrobial Effect Mediated by Cell-Free Supernatants Derived from Probiotics. *Fermentation*. 2022;**8**(7):332. https://doi.org/10.3390/fermentation8070332.
12. Wang Z, Koirala B, Hernandez Y, Zimmerman M, Park S, Perlin DS, et al. A naturally inspired antibiotic to target multidrug-resistant pathogens. *Nature*. 2022;**601**(7894):606–11. [PubMed ID: 34987225]. [PubMed Central ID: PMC10321319]. https://doi.org/10.1038/s41586-021-04264-x.
13. Gideon M, Ladan Z. Synergistic Combinatorial Strategy for Combating Antimicrobial Resistance (AMR) in Clinical Bacteria by Combining Antibiotics with Plant Extracts. *Fine Chem Eng*. 2023:1–12. https://doi.org/10.37256/fce.4120232071.
14. Has IM, Teleky BE, Szabo K, Simon E, Ranga F, Diaconeasa ZM, et al. Bioactive Potential of Elderberry (Sambucus nigra L.): Antioxidant, Antimicrobial Activity, Bioaccessibility and Prebiotic Potential. *Molecules*. 2023;**28**(7). [PubMed ID: 37049862]. [PubMed Central ID: PMC10095832]. https://doi.org/10.3390/molecules28073099.
15. Khonthun C, Khoothiam K, Chumphukam O, Thongboontho R, Oonlao P, Nuntaboon P, et al. Screening and characterization of antioxidant, anti-aging, and anti-microbial activity of herbal extracts in Northern Thailand. *Funct Foods Health Dis*. 2023;**13**(2):52. https://doi.org/10.31989/ffhd.v13i2.1070.
16. Sevindik M, Uysal I. Food spoilage and Microorganisms. *Turk J Agric Food Sci Tech*. 2021;**9**(10):1921–4. https://doi.org/10.24925/turjaf.v9i10.1921-1924.4658.
17. Westover C, Rahmatulloev S, Danko D, Afshin EE, O'Hara NB, Ounit R, et al. Ozone Disinfection for Elimination of Bacteria and Degradation of SARS-CoV2 RNA for Medical Environments. *Genes (Basel)*. 2022;**14**(1). [PubMed ID: 36672826]. [PubMed Central ID: PMC9858956]. https://doi.org/10.3390/genes14010085.
18. Mateescu MC, Grigorescu S, Socea B, Bloanca V, Grigorescu OD. Contribution to the Personalized Management of the Nosocomial Infections: A New Paradigm Regarding the Influence of the Community Microbial Environment on the Incidence of the Healthcare-Associated Infections (HAI) in Emergency Hospital Surgical Departments. *J Pers Med*. 2023;**13**(2). [PubMed ID: 36836443]. [PubMed Central ID: PMC9968232]. https://doi.org/10.3390/jpm13020210.
19. Espinosa-Hernández VM, Morales-Pineda V, Martínez-Herrera E. Skin Infections Caused by Emerging Candida Species. *Curr Fungal Infect Rep*. 2020;**14**(2):99–105. https://doi.org/10.1007/s12281-020-00380-9.
20. Mondal H, Thomas J. A review on the recent advances and application of vaccines against fish pathogens in aquaculture. *Aquac Int*. 2022;**30**(4):1971–2000. [PubMed ID: 35528247]. [PubMed Central ID: PMC9059915]. https://doi.org/10.1007/s10499-022-00884-w.
21. Noga EJ, Smith SA, Ottesen OH. Disease Diagnosis and Treatment. *Practical Flatfish Culture and Stock Enhancement*. Wiley; 2010. p. 259–65.
22. Amenu D. Antimicrobial activity of medicinal plant extracts and their synergistic effect on some selected pathogens. *Am J Ethnomed*. 2014;**1**(1):18–29.
23. Koutchma T. UV Light for Processing Foods. *Ozone Sci Eng*. 2008;**30**(1):93–8. https://doi.org/10.1080/01919510701816346.
24. Romanhole RC, Ataide JA, Moriel P, Mazzola PG. Update on ultraviolet A and B radiation generated by the sun and artificial lamps and their effects on skin. *Int J Cosmet Sci*. 2015;**37**(4):366–70. [PubMed ID: 25720863]. https://doi.org/10.1111/ics.12219.
25. Nichols JA, Katiyar SK. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch Dermatol Res*. 2010;**302**(2):71–83. [PubMed ID: 19898857]. [PubMed Central ID: PMC2813915]. https://doi.org/10.1007/s00403-009-1001-3.
26. Ahmad S, Shabbir A, Tareen N, Ahmad S, Chohan MA. Plant-Derived bioactive compounds as Skin Photo protection Agents. *Int J Nat Med Health Sci*. 2022;**1**(2):46–55. https://doi.org/10.52461/ijnms.v1i2.866.
27. Tacer S, Özüsağlam MA. The Potential Uses of Olive Leaf Extracts in Various Areas. *Eu J Food Sci Tech*. 2022;**6**(1):8–22.
28. Mansur JDS, Breder M, Mansur M, Azulay RD. [Determination of sun protection factor by spectrophotometry]. *An Bras Dermatol*. 1986;**61**(3):121–4. Portuguese.
29. Imam S, Azhar I, Mahmood ZA. 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.). *Asian J Pharm Clin Res*. 2015;**8**(3):234–7.
30. Bambal V, Wyawahare N, Turaskar A, Mishra M. Study of sunscreen activity of herbal cream containing flower extract of Nyctanthes arbortristis L. and Tagetes erecta L. *International Journal of Pharmaceutical Sciences Review and Research*. 2011;**11**(1):142–6.
31. Handali S, Hosseini H, Ameri A, Moghimipour E. Formulation and evaluation of an antibacterial cream from Oxalis corniculata aqueous extract. *Jundishapur J Microbiol*. 2011;**4**(4):255–60.
32. Chen MX, Alexander KS, Baki G. Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. *J Pharm (Cairo)*. 2016;**2016**:5754349. [PubMed ID: 27885352]. [PubMed Central ID: PMC5112321]. https://doi.org/10.1155/2016/5754349.
33. Asan-Ozusaglam M, Gunyakti A. Lactobacillus fermentum strains from human breast milk with probiotic properties and cholesterol-lowering effects. *Food Sci Biotechnol*. 2019;**28**(2):501–9. [PubMed ID: 30956862]. [PubMed Central ID: PMC6431312]. https://doi.org/10.1007/s10068-018-0494-y.
34. Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev*. 2010;**74**(3):417–33. [PubMed ID: 20805405]. [PubMed Central ID: PMC2937522]. https://doi.org/10.1128/MMBR.00016-10.
35. World Health Organization. *Management of drug-resistant tuberculosis: training for staff working at DR-TB management centres: training modules*. World Health Organization; 2014. Contract No.: 9241548991.
36. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. *Microorganisms*. 2021;**9**(10). [PubMed ID: 34683362]. [PubMed Central ID: PMC8541629]. https://doi.org/10.3390/microorganisms9102041.
37. Oliveira I, Sousa A, Morais JS, Ferreira IC, Bento A, Estevinho L, et al. Chemical composition, and antioxidant and antimicrobial activities of three hazelnut (Corylus avellana L.) cultivars. *Food Chem Toxicol*. 2008;**46**(5):1801–7. [PubMed ID: 18316150]. https://doi.org/10.1016/j.fct.2008.01.026.
38. Özaslan M, Oğuzkan SB, Uğraş S, Merve C, Ayşe U, Ülger S, et al.

[Determination of biological activity in hazelnut (Corylus avellana L.) green bark and leaf extracts]. *KSÜ Doğa Bilim Derg*. 2016;**19**(4):373–8. Turkish.

39. Skotarczak K, Osmola-Mankowska A, Lodyga M, Polanska A, Mazur M, Adamski Z. Photoprotection: facts and controversies. *Eur Rev Med Pharmacol Sci*. 2015;**19**(1):98–112. [PubMed ID: 25635982].
40. Ramos S, Homem V, Alves A, Santos L. A review of organic UV-filters in wastewater treatment plants. *Environ Int*. 2016;**86**:24–44. [PubMed ID: 26479831]. https://doi.org/10.1016/j.envint.2015.10.004.
41. Cefali LC, Ataide JA, Fernandes AR, Sanchez-Lopez E, Sousa IMO, Figueiredo MC, et al. Evaluation of In Vitro Solar Protection Factor (SPF), Antioxidant Activity, and Cell Viability of Mixed Vegetable Extracts from Dirmophandra mollis Benth, Ginkgo biloba L., Ruta graveolens L., and Vitis vinifera L. *Plants (Basel)*. 2019;**8**(11). [PubMed ID: 31717792]. [PubMed Central ID: PMC6918343]. https://doi.org/10.3390/plants8110453.
42. Ivanovic S, Avramovic N, Dojcinovic B, Trifunovic S, Novakovic M, Tesevic V, et al. Chemical Composition, Total Phenols and Flavonoids Contents and Antioxidant Activity as Nutritive Potential of Roasted Hazelnut Skins (Corylus avellana L.). *Foods*. 2020;**9**(4):430–44. [PubMed ID: 32260331]. [PubMed Central ID: PMC7230596]. https://doi.org/10.3390/foods9040430.
43. Amadou I, Le G, Shi Y. Evaluation of Antimicrobial, Antioxidant Activities, and Nutritional Values of Fermented Foxtail Millet Extracts byLactobacillus paracaseiFn032. *Int J Food Prop*. 2013;**16**(6):1179–90. https://doi.org/10.1080/10942912.2011.579673.
44. Montella R, Coïsson JD, Travaglia F, Locatelli M, Malfa P, Martelli A, et al. Bioactive compounds from hazelnut skin (Corylus avellana L.): Effects on Lactobacillus plantarum P17630 and Lactobacillus crispatus P17631. *J Funct Foods*. 2013;**5**(1):306–15. https://doi.org/10.1016/j.jff.2012.11.001.