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INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF BLUEBERRY TWIG EXTRACT AND ITS POTENTIAL USE IN COSMETIC FORMULATIONS

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Topical infections and their difficult treatments are one of the challenges arising from antimicrobial resistance. Herbal formulations are an important resource in drug development for the treatment of infections that cause various health problems. Therefore, this study aimed to determine the antimicrobial and photoprotective potential of blueberry (*Vaccinium spp.*) twig extract (BTE) as well as formulate a herbal cream for topical treatments. The biological activity of BTE against clinically important strains such as *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* RSKK 863, *Candida albicans* ATCC 10231 and *Candida glabrata* RSKK 04019 was evaluated using both disc diffusion and micro-dilution methods. BTE at a concentration of 4 mg/µL produced the highest inhibition zone diameter of 11.01 mm on *B. cereus* RSKK 863, followed by *E. coli* O157:H7 with 10.42 mm. The results from micro-dilution methods indicated that the lowest minimum inhibitory concentration (MIC) value (25 µg/µL) was against *B. cereus* RSKK 863. The protective capacity of BTE against UV-B rays was determined spectrophotometrically. The sun protection factor (SPF) of the extract was calculated as 4.60 and in cream formulations containing 10 mL of this extract, SPF increased to 26.12. Moreover, in cream formulations where BTE was combined with *Streptococcus thermophilus* MAS-1, a synergistic antimicrobial effect was observed with wider zones of inhibition against all test microorganisms. Based on the results, BTE may be suitable for evaluation as an antimicrobial, antifungal and photoprotective agent in multifunctional topical formulations containing natural ingredients.

Keywords: antimicrobial, blueberry, extract, probiotic, herbal creams

1. Introduction

Plant-based compounds have been among the primary natural resources used for treatment over the ages. Today, these natural products are of great interest, especially in pharmaceutical research, in terms of discovering new drug candidates [1]. Natural compounds obtained from plants constitute the precursors of many modern drugs due to their structural diversity and biological activities [2]. Medicinal plants, widely used in traditional medical systems, offer numerous therapeutic properties such as antimicrobial, antioxidant and anti-inflammatory [3]. Blueberry (*Vaccinium spp*.) extracts are rich in phenolics and flavonoids with potent antioxidant properties. Recent studies suggest that these extracts may help protect human keratinocyte (HaCaT) cells against oxidative stress [4].

The blueberry (*Vaccinium spp*.) genus is of special importance among medicinal plants. Although it is generally known for its fruit, new research shows that blueberry twigs also offer a rich phenolic content and high antioxidant capacity [5],[6]. Blueberries contain many bioactive components such as phenolic compounds, anthocyanins, flavonoids, tannins, ferulic acid, gallic acid, vanillic acid and chlorogenic acid [7]. Blueberry twig extracts (BTEs) are particularly rich in chlorogenic acid and various flavonoids, moreover, have the potential to keep skin healthy by exhibiting antimicrobial, anti-inflammatory and free radical scavenging properties [8],[9]. As the largest organ of the body, skin is both a barrier against the external environment and a structure that bears the most visible signs of aging. Long-term exposure to UV rays increases the risk of skin cancer and accelerates skin aging [10]. Long-term use of chemical sunscreens can have negative effects on skin biology and drives the search for alternative natural solutions [11],[12]. Active compounds obtained from plants with the help of organic solvents are widely used in the food, cosmetics and pharmaceutical industries [13]. Sunscreen products containing herbal extracts have become an important area of research because they not only provide photoprotection but also have a lower side effect profile [14].

On the other hand, skin infections constitute an increasing health problem worldwide. The problem of

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resistance to antibiotics increases the need for new antimicrobial agents [15],[16]. The antimicrobial effects of phenolic compounds are associated with various mechanisms such as increasing cell membrane permeability, protein denaturation, enzyme inhibition and binding to microbial DNA [17],[18]. For this reason, plant-derived antimicrobial agents have come to the fore in current research.

Probiotics are live microorganisms that exhibit positive effects on the health of the host when taken orally in suitable amounts [19]. Probiotics create a protective barrier against infections and keep skin healthy by maintaining skin microbiota in balance [20]. They help maintain skin health as well as moisturize it by using probiotic microorganisms and active ingredients, thereby contributing to the development of environmentally-friendly cosmetic ingredients [21]. *Streptococcus thermophilus* has the capacity to produce bacteriocins during fermentation and these antimicrobial peptides can suppress pathogen proliferation on the skin [22]. The combination of plant extracts and probiotics both strengthens the natural antimicrobial effect and increases the functionality of topical products [23].

The microorganisms used in antimicrobial studies conducted with blueberry twigs are among the main causes of common skin infections in humans. For example, *Staphylococcus aureus* is frequently seen in both community- and hospital-acquired infections, causing skin and soft tissue infections such as impetigo, cellulitis and furuncles [24]. Although *Staphylococcus epidermidis* is generally considered a harmless member of the skin flora, it can cause opportunistic infections in the presence of wounds or invasive medical devices [25]. Although *Escherichia coli* O157:H7 is known as an enteric pathogen, it carries the risk of secondary infections through skin contamination in traumatic injuries [26]. *Pseudomonas aeruginosa* is an important pathogen that grows particularly well in moist environments as well as can cause infection in burn wounds and chronic ulcers due to its resistant structure [27]. *Candida albicans* causes fungal infections in areas where warm and moist skin is found, leading to clinical conditions such as intertrigo and Candidal dermatitis [28]. The mechanisms of antibiotic resistance developed by these pathogens against classical antibiotics have rendered the research of natural and plant-derived antimicrobial agents important [29],[30].

Waste generated as a result of product processing in the agricultural and food industries causes significant problems such as environmental pollution, resource waste and greenhouse gas emissions [31]. Therefore, the reuse of agricultural waste by evaluating it to produce bioactive compounds offers a sustainable approach both economically and environmentally.

In this study, the potential of blueberry twig extract in pharmaceutical and cosmetic applications was investigated. The antimicrobial effects of BTE on test microorganisms were investigated. Sun protection factor (SPF) values BTE as well as BTE+commercial cream mixtures were measured spectrophotometrically and their potential for use as natural sunscreens investigated. Finally, the antimicrobial effects of cream formulations containing BTE and *S. thermophilus* MAS-1 on test microorganisms were determined as well as the synergistic effect of their combination evaluated.

2. Experimental

2.1. Plant material

Blueberry twigs were collected from Alata Horticulture Research Institute (Mersin, Türkiye) in June 2021.

2.2. Preparation of a water extract of blueberry twigs

The collected blueberry twigs were first washed with distilled water and then dried in a well-ventilated environment away from direct sunlight. The completely dried twigs were ground using a Waring blender. The powders obtained were stored in a cool and dark environment to protect them from light and moisture.

For the extraction process, the powdered plant material was treated with distilled water in a hot water bath (75°C) for 2 days, 6 hours per day. The obtained water-based extract was then evaporated to remove the solvent. In the final step, the blueberry twig water extract (BTE) was dissolved in dimethyl sulfoxide (DMSO) and sterilized using a filter with a pore diameter of 0.45 µm.

2.3. Test microorganisms

In the studies, active cultures of *E. coli* O157:H7, *S. aureus* ATCC 25923 and *Bacillus cereus* RSKK 863 were grown at 37°C in Nutrient Broth (NB)/Agar. *C. albicans* ATCC 10231 and *Candida glabrata* RSKK 04019 were grown at 30°C in Yeast Extract Peptone Dextrose (YPD)/Agar. The test microorganisms were prepared by adjusting to the 0.5 McFarland standard (1×10^8 CFU/mL).

2.4. Evaluation of the antimicrobial activity of BTE

2.4.1. Disc diffusion method

The disc diffusion method was utilized to evaluate the antimicrobial activity of blueberry twig extract (BTE). Each microorganism suspension (100 µL) was spread onto the surface of appropriate solid media. Having placed sterile discs (Whatman No: 1, diameter: 6 mm) on the surface of the agar medium, 10 and 20 µL of BTE were applied to each disc. Each application was repeated three times [32]. As positive controls, standard antibiotic discs were used, namely Fluconazole (FLU, 25 µg/disc), Linezolid (LNZ, 30 µg/disc) and Kanamycin (K, 30 µg/disc). The sterilized distilled water served as a negative control. The Petri dishes were incubated for 24 hours under appropriate conditions for each microorganism. After the incubation period, the diameters of the inhibition zones formed around the discs

were measured using calipers and the average values of the obtained data reported.

2.4.2. Microdilution technique

A microdilution technique was applied to determine the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) of BTE [33]. For this purpose, the microorganism suspensions adjusted to the 0.5 McFarland standard were added to the diluted BTE (12.50 - 200 µg/µL) obtained following serial two-fold dilutions incubated for 24 hours under appropriate conditions for each microorganism. After incubation, the lowest extract concentration at which inhibited microbial growth occurred was recorded as the MIC value. Then, the samples from tubes containing diluted BTE were inoculated using the drop-plate technique and incubated at appropriate temperatures for 24 hours. The lowest concentrations at which no microbial growth was observed after incubation were recorded as the MBC or MFC values.

2.5. Determination of the sun protection factor

The effect of BTE as a sunscreen was determined by a spectrophotometric method [34]. BTE was homogenized by dissolving it in ethanol (96%) at a concentration of 2 µg/µL. The absorbance values of the three separate samples were measured using a spectrophotometer (Beckman Coulter Inc., USA) at 5 nm intervals between the wavelengths of 290 and 320 nm. These measured values were evaluated in the SPF calculation using equation developed by Mansur et al. [35].

The effect of BTE as a sunscreen was analyzed not only as an extract but also by co-formulating it with a commercial cream. BTE (5% w/v) and commercial cream (10% w/v) were mixed before adding distilled water to achieve the final volume. A sample (0.1 g) was taken from the resulting mixture and homogenized in 40% (v/v) ethanol (10 mL) before being ultrasonicated (Hielscher UP100H; amplitude = 100%) for 5 mins. The absorbances of the prepared samples in volumes of 2.5, 5.0 and 10.0 mL were measured and the SPF values calculated with the help of the following Mansur equation (Mansur et al.) [35]:

$$SPF = CF \; x \; \sum_{290}^{320}[EE(\lambda) \; x \; I(\lambda) \; x \; Abs(\lambda)] \qquad (1),$$

where:

CF:	correction factor (10),
EE(λ):	erythemal effect,
I(λ):	sunlight intensity at a given wavelength,
Abs(λ):	optical density (OD) of BTE at a given wavelength,
EE(λ) × I(λ):	constant coefficient as stated by Sayre et al. [36].

2.6. Evaluation of the antimicrobial activity of a cream developed with BTE and a probiotic

The antimicrobial activities of cream formulations developed with BTE and *S. thermophilus* MAS-1 were evaluated using the methods previously described by Chen et al. [37] with some modifications. A commercial cream (10% w/v) and BTE (20% w/v) were used in the formulations. A *S. thermophilus* MAS-1 culture (OD_{600nm}; 1.6) was added to groups containing live probiotic bacteria and distilled water added to achieve the same volume in control groups without bacteria. No extract or bacterial culture was found in the negative control group. The formulations were sterilized by filtration using a sterile syringe filter with a pore diameter of 0.45 µm before being stored in a dry and dark location at 4 °C until analyzed.

The antimicrobial activities of cream formulations were evaluated using the well diffusion method [38]. For this purpose, 100 µL of each bacterial suspension adjusted to the 0.5 McFarland standard was spread onto the surface of a solid medium. 100 µL of the cream samples were added to the wells in each Petri dish before being incubated for 24 hours. After incubation, the inhibition zones formed around the well were measured with calipers and their diameters recorded in mm. Each application was triplicated and the average values of the obtained data reported.

2.7. Statistical analysis

Data are presented as the mean and standard deviation (SD) of three separate measurements. Statistical analyses were performed using GNU PSPP software. Two-way Analysis of Variance (ANOVA) was used to determine significant differences between groups and significant results were analyzed using the Tukey's post-hoc test. The p value of <0.0001 was considered statistically significant.

3. Results and discussion

Today, the excessive and unintentional use of antibiotics has caused a serious increase in antimicrobial resistance rates in healthcare [29],[30]. The treatment of infections caused by antibiotic-resistant microorganisms has become both time-consuming and clinically complex. As a result, it is necessary to develop safe, natural and effective bioactive compounds that can prevent or control the growth of pathogenic microorganisms. Over recent years, plant-based extracts in particular have come to the forefront as natural alternatives that can replace synthetic chemicals in pharmaceutical and cosmetic applications [30]-[39]. These plant-based ingredients with antimicrobial properties are important because they are less detrimental to human health and promote environmental sustainability [40]. In the cosmetics sector, the use of plant extracts together with probiotics increases the antimicrobial effectiveness of products and

Table 1: Disc diffusion test results of BTE on test microorganisms

Test microorganisms	Inhibition zone diameter (mm±SD)				
	BTE (2 mg/µL)	BTE (4 mg/µL)	K (30 µg/disc)	LNZ (30 µg/disc)	FCA (25 µg/disc)
E. coli O157:H7	7.36±1.20^{a}	10.42±0.49^{b}	20.83±0.67^{c1}	8.61±0.32^{abd1}	NA^{e}
S. aureus ATCC 25923	7.43±1.25^{a}	9.52±0.87^{a}	23.28±0.30^{b2}	26.32±1.10^{b2}	NA^{c}
C. glabrata RSKK 04019	6.46±0.34^{a}	9.36±0.71^{b}	26.51±1.59^{c2}	19.28±0.53^{d3}	NA^{e}
C. albicans ATCC 10231	8.38±1.27^{a}	9.88±0.25^{a}	25.63±2.33^{b2}	20.92±1.17^{c3}	NA^{d}
B.cereus RSKK 863	7.86±0.67^{a}	11.01±0.86^{b}	26.64±2.87^{c2}	31.57±1.43^{d4}	NA^{e}

*BTE: extract, K: Kanamycin, LNZ: Linezolid, FCA: Fluconazole, NA: No activity
*When the two-way ANOVA was applied and then the Tukey post-hoc test performed, the superscripted letters in the rows and the superscripted numbers in the columns differed in value ($p<0.0001$).
*Row: F(Sig): 4.50(1376), Column: F(Sig): 4.50(60.16).

meets the need for natural preservatives by enriching the variety of functional content [20]-[23].

3.1. Antimicrobial activity of BTE

The disc diffusion test results showed that 2 mg/µL of BTE yielded an inhibition zone diameter between 6.46 (*C. glabrata* RSKK 04019) and 8.38 mm (*C. albicans* ATCC 10231) on the test microorganisms, while the values obtained were statistically insignificant ($p>0.0001$). 4 mg/µL of BTE created the largest inhibition zone diameter on *B. cereus* RSKK 863 (11.01 mm) followed by *E. coli* O157:H7 (10.42 mm) ($p>0.0001$). The inhibition zone diameters on microorganisms increased as the BTE concentrations rose. As the extract concentration increased, the increase in the inhibition zone diameter was statistically significant in all microorganisms except for *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 ($p<0.0001$). The disc diffusion test results revealed that BTE exhibited increasing inhibitory effects against both bacteria and yeast species in a dose-dependent manner (*Table 1*). These findings indicate that the extract exhibits a broad spectrum of antimicrobial properties not only against the bacterial strains but also against the tested yeast species.

Yavorska et al. [6] reported that *Vaccinium corymbosum* shoot extracts produced inhibition zones of up to 12 mm in diameter against *C. albicans* under ethanol extraction. In this study, water-based BTE (prepared with distilled water) inhibition zones with a diameter of 15.10 mm on *B. cereus* and 11.30 mm on *C. albicans* were produced**.** These results may be related to climate conditions, the extraction solvent and method used. In some studies in the literature, *Vaccinium* species are rich in various anthocyanins, which may explain their more effective antimicrobial effect on the test microorganisms [41],[42].

Table 2: Micro-dilution test results of BTE

Test microorganisms	Micro-dilution test	
	MIC (µg/µL)	MBC or MFC µg/µL)
E. coli O157:H7	50	50
S. aureus ATCC 25923	50	50
C. glabrata RSKK 04019	50	50
C. albicans ATCC 10231	100	100
B. cereus RSKK 863	25	100

The micro-dilution method showed that BTE exhibited the lowest MIC value (25 µg/µL) on *B. cereus* RSKK 863 and this bacterium emerged as the most susceptible strain of the extract. *C. albicans* ATCC 10231 was determined as the most resistant microorganism to the extract with MIC and MFC values of 100 µg/µL. These results show that the antimicrobial activity of BTE varies according to the structure of the cell wall and strain of the microorganism (*Table 2*). As a result, it is thought that BTE has the potential to be used as a natural antimicrobial agent in topical products due to its good antimicrobial and antifungal properties.

In this study, the water-based BTE extract exhibited an inhibitory effect at a lower concentration with a MIC value of 25 µg/µL against the same microorganism, suggesting that the water-based form of BTE may be more effective due to the extraction method used or the soluble phenolic compound profile. Redondo-Blanco et al. [8] reported MIC values ranging from 125–250 µg/µL in cranberries and blueberries (*Vaccinium spp.*) against *C. albicans* strains. In our study, an MIC value of 100 µg/µL was recorded against the same microorganism, which is lower than those reported in the literature and shows that BTE is also effective on yeast species. In addition, MIC values of 50 µg/µL were observed in *E. coli* O157:H7, *S. aureus* ATCC 25923 and *C. glabrata* RSKK 04019 strains, indicating that the water-based BTE has a broad-spectrum antimicrobial potential against both Gram-negative and Gram-positive bacteria.

3.2. Solar protection activity of BTE and cream mixtures

Long-term exposure to sunlight is one of the main causes of health problems such as skin aging, photodermatoses and skin cancer [11],[12]. Therefore, the development of

Table 3: SPF of BTE

λ (nm)	*Abs*	*CF x EE(λ) x I(λ) x Abs(λ)*
290	0.71	0.10
295	0.60	0.49
300	0.52	1.51
305	0.44	1.46
310	0.37	0.69
315	0.32	0.26
320	0.28	0.05
		SPF Value = 4.60±0.02

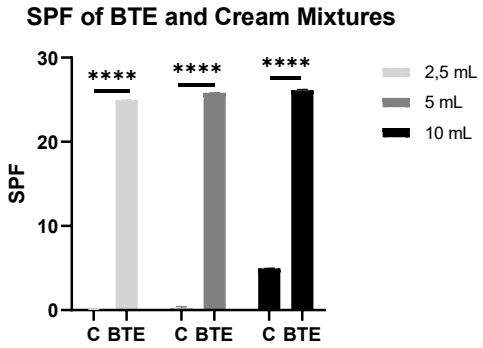

Figure 1: SPF of BTE and cream mixtures

*When one-way ANOVA was applied and then a Tukey post-hoc test performed, the differences in the values of the superscripted letters in the rows and the superscripted numbers in the columns were p<0.0001.
*Row: F(Sig): 1.12(243609).

products that are applied to the skin and provide protection by absorbing UV-B rays is of great importance. The undesirable effects of chemical filters in sunscreens, such as potential toxicity and skin irritation, have increased the interest in herbal ingredients [13], [42]. The photoprotective effects of herbal extracts are associated with the ability of compounds such as polyphenols, flavonoids and phenolic acids to absorb UV rays as well as their antioxidant activities [39]. The SPF values of BTE-containing formulations were determined by in vitro spectrophotometric methods. The SPF value of BTE (2% ethanol solution) was determined to be 4.60 (*Table 3*). Furthermore, the SPF values obtained by mixing BTE with the commercial cream in different ratios (2.5, 5.0 and 10.0 mL) were determined to be 24.92, 25.80 and 26.12, respectively (*Figure 1*). It was observed that SPF values increased at a similar rate with increasing BTE concentration (2.5, 5.0 and 10.0 mL) and the values obtained were statistically significant ($p<0.0001$). Therefore, BTE can provide protection against UV-B rays even at low concentrations. These results indicate that the photoprotective effect of the bioactive compounds with antimicrobial potential present in BTE is enhanced by increasing the concentration.

Bucci et al. [43] evaluated the protective effects of ethanol extracts obtained from blueberries on human dermal fibroblasts exposed to UV-B radiation. They suggested that ethanol extracts can be used as active compounds that protect the skin against UV-B-induced skin photoaging by preventing DNA damage in cells as well as suppressing collagen degradation and cellular functions such as photoaging. Franco et al. [44] reported that the nanocapsule formulation prepared from *V. myrtillus* (blueberry) fruit achieved an SPF value of 34.6 in vitro. Similarly, it was reported that the extract obtained from *V. uliginosum* (bog blueberry) fruit wax exhibited significant photoprotective effects thanks to its high phenolic and antioxidant content [45]. Compared to these studies, in this study, the water-based blueberry twig extract (BTE) exhibited an SPF value of 4.60 in its crude form. However, when formulated with the commercial cream, it achieved SPF values of 24.92, 25.80 and 26.12 depending on the amount of extract added. These results show that BTE has a good UV-B absorption capacity and provides effective photoprotection in the absence of any chemical filter or nanoencapsulation. In addition, the fact that BTE is obtained from the twigs of the plant, which is usually considered to be waste, increases the potential of this extract as a sustainable and environmentally-friendly topical photoprotective agent. Franco et al. [44] achieved a high level of photoprotective activity by reaching an SPF value of 34.6 with the formulation they prepared from *V. myrtillus* fruit extract using the nanoencapsulation method. However, Nguven et al. [45] reported that the extract obtained from *V. uliginosum* fruit wax also exhibited significant photoprotective effects against UV-B rays. However, fruit parts were used in both studies and the effectiveness of the extracts generally increased in the presence of nanocarriers or lipophilic carriers. In this study, only the water-based blueberry twig extract (BTE) achieved an SPF value of 26.12 in a formulation containing 10% extract without any encapsulation or a chemical UV filter. As seen in *Figure 1*, as the BTE ratio increased, the SPF value also increased linearly, revealing that the capacity of BTE to absorb UV-B rays is dose-dependent. These findings indicate that BTE can be an effective natural agent in itself or as an adjunct ingredient in topical sunscreen products.

3.3. Antimicrobial activity of cream formulations containing BTE and a probiotic

The increasing prevalence of antimicrobial resistance has increased interest in alternative treatment approaches for skin infections. Herbal extracts and probiotic microorganisms stand out as natural and safe options in this regard [20]. The antimicrobial activities of cream formulations containing BTE and *S. thermophilus* MAS-1 were investigated in vitro and the results are presented in *Table 4*. In the control group, the inhibitory effect of only applying cream (C) was determined to be 4.91 mm

on *S. aureus* ATCC 25923 and 3.15 mm on *C. albicans* ATCC 10231 ($p<0.0001$), while no inhibition was observed against other strains ($p>0.0001$). The cream formulation containing BTE (C+BTE) exhibited significant antimicrobial activity against all microorganisms (3.86-9.84 mm zone of inhibition) with the largest inhibition zones recorded as 6.84 mm against *S. aureus* ATCC 25923 and 6.39 mm against *C. albicans* ATCC 10231 ($p>0.0001$). *S. thermophilus* MAS-1 with the commercial cream (C+P) samples yielded a limited inhibition zone diameter of 6.63 mm against *S. aureus* ATCC 25923 and 5.14 mm against *C. albicans* ATCC 10231, moreover, the difference between them was not significant ($p>0.0001$). The most striking results were obtained with the cream formulation containing both BTE and probiotics (C+P+BTE). The combined sample produced the largest inhibition zones against all the microorganisms and exhibited a synergistic effect. With the synergistic effect of the extract and probiotic strain, an increase in antimicrobial activity was observed with an inhibition zone of 8.15 mm for *S. aureus* ATCC 25923 and 7.99 mm for *B. cereus* RSKK 863. These findings suggest that the phenolic compounds found in BTE exhibit broad-spectrum antimicrobial activity and using them in conjunction with probiotics can significantly enhance this activity.

Kostov et al. [23] reported that the synergistic antimicrobial effect increased in cosmetic formulations prepared by using herbal extracts and probiotics together, moreover, that such combinations also promoted product stability. Similarly, Rawal and Ali [20] emphasized that probiotics such as *S. thermophilus* form a natural defense barrier against pathogenic microorganisms on the surface of the skin through bacteriocin production. In this study, minimal levels of inhibition were observed in the negative control group containing only cream; a zone diameter of 4.91 mm was formed in the *S. aureus* strain and 3.15 mm in the *C. albicans* strain. These values increased significantly in the formulation containing BTE and were measured to be 6.39 mm for *C. albicans* and 5.47 mm for *B. cereus*. Limited inhibition was also observed in the cream group containing only probiotics (*S. thermophilus* MAS-1). However, the highest antimicrobial effect was obtained in the combined formulation where BTE and a probiotic were used together. This combination created inhibition zones of 6.22 mm in diameter in the *C. albicans* ATCC 10231 strain and 7.99 mm in the *B. cereus* RSKK 863 strain. These results show that plant phenolic compounds and probiotic bacteria originating from human milk can significantly increase antimicrobial activity by exhibiting a synergistic effect. When compared with the zone diameters in similar studies reported in the literature, these values are remarkable and show that the BTE+probiotic combination can be an effective and natural topical agent against clinically important pathogens.

4. Conclusions

In this study, blueberry twig extract (BTE) exhibited antimicrobial and antifungal activity against both bacterial and fungal pathogenic microorganisms. Furthermore, the addition of BTE to cream formulations significantly increased the sunscreen capacity of the formulations. These findings indicate that adding BTE to commercial creams can bring about both antimicrobial and photoprotective properties to formulations. The combination of BTE with *S. thermophilus* MAS-1 originating from human milk exhibited a synergistic effect in cream formulations, resulting in broad-spectrum antimicrobial activity. In this context, probiotic-supported topical formulations containing BTE stand out as effective natural candidates for preventing infections, maintaining healthy skin and protecting against the harmful effects of sunlight. In light of the data obtained, it was concluded that BTE has the potential to be used as a natural additive in the pharmaceutical and cosmetic sectors, moreover, may offer an environmentally-friendly alternative to synthetic components.

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