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Posted Date: 24 March 2026

doi: 10.20944/preprints202603.1791.v1

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Antioxidant, Photoprotective, and Antimicrobial Potential of Oil Extract of *Usnea barbata* L. F.H.Wigg from Călimani Mountains, Romania

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Abstract

In order to enhance the value and stability of vegetable oils, they may be enriched with essential oils and plant extracts with strong antioxidant activity, yielding innovative formulations with potential applications in skincare. The present research aims to investigate the bioactive properties of an *Usnea barbata* extract in Jojoba oil (JO) enriched with 5% Peppermint oil (PEO), and 10% vitamin E (PJO). The oil extract (UBPJO) was obtained through cold maceration. The UBPJO antioxidant activity was investigated using 2 methods (DPPH and ABTS). The sunscreen capacity of UBPJO was evaluated by measuring their sun protection factor (SPF) values. The antimicrobial potential was investigated against *S. aureus, E. coli,* and *C. albicans.* The antioxidant activity of UBPJO was 1.5 times higher than PJO. Consequently, the sunscreen efficacy (assessed by SPF measurements) indicated satisfactory UVB protection abilities against *S. aureus* (UBPJO vs. PJO: 32.41 vs. 30.58). UBPJO exhibited significantly greater inhibitory effects compared to PJO against *S. aureus* (MIC = 18.75 ± 6.25 vs. 37.50 ± 0.00, $p < 0.05$). and *C. albicans* (9.62 ± 2,87 vs. 37.5 ± 12.5, $p < 0.05$). The bioactive properties investigated in the present study support the inclusion of UBPJO in various skin protective formulations with antiaging, antibacterial, and antifungal effects.

Keywords: *Usnea barbata*; jojoba oil; peppermint essential oil; vitamin E; antioxidant activity; photoprotective effect; antimicrobial potential

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1. Introduction

Achieving and maintaining healthy skin involves a comprehensive approach that incorporates daily protection, targeted skincare routines, and mindful lifestyle choices [1]. One of the most significant contributors to visible skin aging is UV exposure, which accounts for 80–90% of the signs of aging [2–4].

Exposure to ultraviolet (UV) radiation is a major stress factor for the skin, directly affecting epidermal cellular structure and function and often causing various skin cancers. Ultraviolet B (UVB) radiation, with wavelengths between 280 and 320 nm, triggers an acute inflammatory response of the skin, mainly mediated by proinflammatory substances derived from arachidonic acid (prostaglandins and leukotrienes)[5]. It is mainly responsible for erythema, skin alterations, and even carcinogenesis [6–9]. On the other hand, UVA radiation, spanning 320-400 nm, penetrates more deeply, leading to the development of skin photoaging, characterized by reduced elasticity, skin tone loss, collagen breakdown, and wrinkles [10,11].

According to literature data, chronic exposure to UV radiation results in oxidative stress via ROS generation, DNA damage, hyperpigmentation, premature photoaging, and increases the long-term risk of melanoma and non-melanoma skin cancers [12,13]. Therefore, making daily sun protection a non-negotiable part of a skincare regimen is essential. This can include using a broad-spectrum sunscreen with at least SPF 30, as well as incorporating antioxidants, moisturizers, and antimicrobials, which can enhance overall skin health and resilience against aging [14,15]. Most commercial formulations contain chemical or mineral filters that can cause irritation, sensitization, or environmental harm [16–22]. Therefore, modern consumers are increasingly aware of the potential health risks posed by synthetic chemicals in personal care products [23,24], driving a significant shift toward natural, eco-friendly cosmetic formulations. This trend has spurred extensive scientific research into plant-derived compounds that can serve as functional ingredients in cosmetics — offering not only aesthetic benefits but also health-promoting effects, including sunburn protection and antioxidant and antimicrobial properties [25–29].

Various cosmetic ingredients can be identified using the International Nomenclature of Cosmetic Ingredients (INCI), which provides a standardized system to ensure clarity and consistency in the cosmetics industry. It contains over 30,000 INCI names, chemical structures, synonyms, and regulatory references. The International Cosmetic Ingredient Dictionary and Handbook, where these names are listed, is also available electronically as wINCI, making it easily accessible for those in the field. This system enhances transparency and fosters trust among consumers and manufacturers alike, and is available online at https://www.personalcarecouncil.org/resources/inci/. The European Commission official Database (CosIng, available online at https://ec.europa.eu/growth/tools-databases/cosing/) includes over 15,000 ingredients and provides details on the INCI name, CAS number, ingredient functions, and possible restrictions on use under Regulation (EC) No 1223/2009. As an initiative of the European cosmetics industry, COSMILE Europe provides transparent, easy-to-understand information for consumers about the safety and role of each ingredient, available online at https://cosmileeurope.eu/. It contains approximately 30,000 cosmetic ingredients. A plant-derived product with multiple benefits for skincare, included in the above-mentioned databases, is a lichen extract (*Usnea barbata* extract) available online at https://cosmileeurope.eu/inci/detail/16750/usnea-barbata-extract/. Over six years (2016 – 2022), as part of a doctoral and postdoctoral project, our team investigated the composition and bioactivities of *U. barbata* harvested from an unpolluted zone in the Calimani Mountains. *U. barbata* extracts were obtained in various solvents (ethanol, methanol, ethyl-acetate, acetone, and Canola oil) through different methods (maceration and Soxhlet extraction). Usnic acid and other phenolic secondary metabolites were identified and quantified through UHPLC (caffeic acid, p-coumaric acid, ellagic acid, chlorogenic acid, cinnamic acid, and gallic acid) [30–35]. We also isolated usnic acid from the dry *U. barbata* extract in ethyl acetate by semi-preparative chromatography [36], and validated a UHPLC method for the determination of usnic acid content in *U. barbata* extract in Canola oil [35]. Moreover, the most active extracts were incorporated in mucoadhesive oral films with potential applications in oral cancer [37–40].

Based on our previously published results, *U. barbata* harvested from the same unpolluted zone of the Calimani Mountains in the same period was extracted in a mixed oil solvent, Jojoba oil (JO) enriched with 5% Peppermint essential oil (PEO) and 10% vitamin E. We recently identified the bioactive constituents (using the GC-MS method), determined the total phenolic content (including usnic acid and other phenolic acids), and investigated the physicochemical properties of this complex oil extract, with potential applications in the cosmetic field [41]. Our published results indicated that the combination of all four ingredients (UBPJO) creates a multifunctional cosmetic system in which each ingredient contributes complementary properties (Table 1) [41].

Table 1. Contributions and synergistic roles of all four constituents of *U. barbata* oil extract, and the main phytoconstituents responsible for UBPJO pharmacological properties.

Ingredient	Primary Contribution	Synergistic Role	References
Jojoba Oil	Carrier, emollient, skin barrier support	Stable lipid matrix that protects and delivers other actives	[42]
Peppermint Oil (5%)	Antimicrobial, cooling, antioxidant	Adds volatile compounds; enhances penetration via cooling/vasodilatory effect	[43–49]
Vitamin E (10%)	Lipid-soluble antioxidant, photoprotection	Regenerates other antioxidants; protects unsaturated components from oxidation	[50,51]
***U. barbata* extract**	Phenolic-rich, antioxidant, antimicrobial, and potential UV-filter	Strongly boosts TPC; extends oxidative stability; adds lichen-specific bioactive metabolites	[5,52–57]

Main constituents	**Samples**		
	PJO [41]		**UBPJO [41]**
TPC (µg GAE/g)	247.56		297.27
Volatile constituents (%)	PEO [58]	PJO [41]	UBPJO [41]
L-Limonene	2.08	9.93	7.11
(+/-)-Linalool	0.06	0.11	-
L-menthone	-	3.94	2.97
(-)-isomenthone / cis-p-Menthan-3-one	26.52	18.34	19.50
Methyl chavicol/ estragole	-	8.99	7.81
trans-Carveol	-	1.10	0.80
Neoisomenthol	55.09	-	-
Eucalyptol	5.04	-	-
(+)-Pulegone	-	29.93	41.66
Carvone	-	0.64	1.38
8-Hydroxy-p-menthan-3-one	-	1.34	1.62
Limonene-1,2-diol/ Limonene glycol	-	1.33	0.52
Methyleugenol	-	1.63	0.95
trans-Caryophyllene	1.61	0.81	0.51

TPC – Total phenolic content; PJO—Jojoba oil with 5% Peppermint Essential Oil and 10% Vitamin E; UBPJO—*U. barbata* oil extract.

The addition of *U. barbata* extract to the already antioxidant-rich base of Jojoba oil, Peppermint oil, and Vitamin E significantly increased the total phenolic content. This suggests a true additive-to-synergistic enhancement of antioxidant capacity, because:

- Vitamin E (tocopherol) contributes lipid-soluble radical-scavenging activity
- Peppermint oil contributes terpenes with antioxidant properties
- Jojoba oil provides a stable, oxidation-resistant liquid wax matrix
- *U. barbata* is rich in unique lichen secondary metabolites with phenolic structure (e.g., usnic acid, depsides, depsidones) that substantially boost the total phenolic content [30,33,59–62].

doi:10.20944/preprints202603.1791.v1

AFM and FTIR analyses confirmed the physicochemical compatibility of the combined ingredients [41]:

- AFM revealed surface roughness differences between PJO and UBPJO, indicating that *U. barbata* extract integrates into the oil matrix and modifies its microstructure;
- FTIR confirmed the chemical integrity of the combined formulation, showing no destructive interactions between components.

Therefore, the combination has a higher oxidative stability [41], suggesting that *U. barbata* phenolics synergize with Vitamin E to quench free radicals more effectively, Jojoba oil's inherent stability (as a liquid wax ester rather than a triglyceride) provides a protective matrix that slows oxidative chain reactions [63,64], and Peppermint oil's volatile antioxidants contribute an additional layer of protection [44,65].

This complex formulation also showed improvements in rheological properties [41]. These aspects are relevant because they affect the bioavailability of active compounds at the skin surface — a more spreadable formulation ensures better contact time and penetration of the antioxidant, antimicrobial, and UV-protective compounds.

All previous findings support the conclusion that the four ingredients work synergistically rather than merely additively, making the combined formulation a promising candidate for natural, multifunctional skincare products.

As an innovative approach in developing natural skin care products, UBPJO-based formulations are expected to combine emollient, antioxidant, and antimicrobial effects with slightly enhanced UV protection. Thus, the present study aims to investigate the antioxidant activity, sunscreen properties, and antimicrobial potential of *U. barbata* extract in JO enriched with 5% Peppermint oil and 10% vitamin E.

2. Results

2.1. Antioxidant Activity

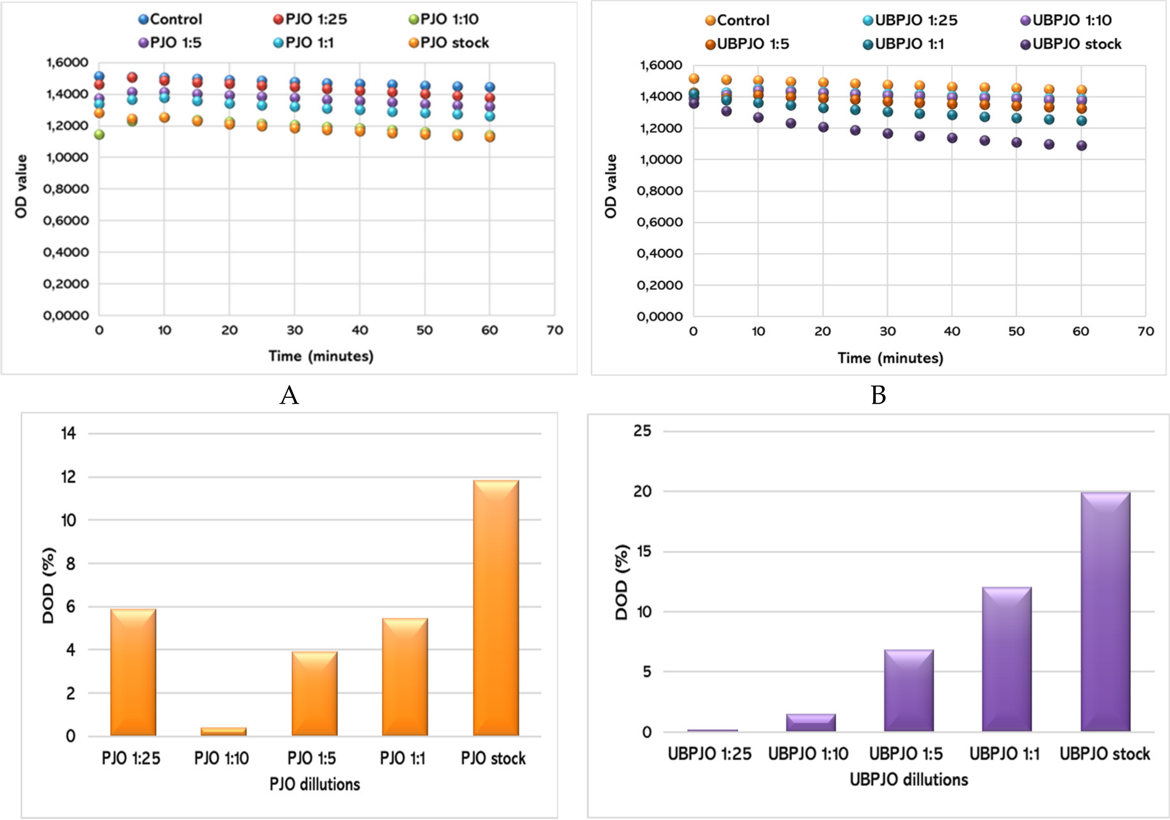
To accurately evaluate the antioxidant activity of PJO and UBPJO, the samples were diluted in methanol (1:1, 1:5, 1:10, and 1:25) and compared with a solvent-based standard.

2.1.1. DPPH Method

The in vitro radical-scavenging capacity of PJO and UBPJO was assessed using the DPPH method, with both kinetic and endpoint measurements (Figure 1).

PJO has an OD trajectory similar to that of UBPJO (Figure 1A and 1 B). However, the ΔOD values are lower than those of UBPJO (Figure 1C and D). The highest PJO dilution (1:25) has the second-most intense antioxidant effect after the stock solution (ΔOD = 5.87% vs. 11.87%, Figure 1C).

Our results show that the standard OD dynamics was 4,74%. For the UBPJO, the percentage of variation was almost insignificant (0,19% for the 1:25 dilution and 1,50% for the 1:10 dilution), but became highly significant for the 1:5, 1:1, and undiluted samples. (6,87%, 12,03%, and 19,92% respectively). This shows that the antioxidant effect of the UBPJO is maintained up to 1:5 but diminishes after 1:10. Overall, the ΔOD values for UBPJO were 1.5 times higher than PJO (Figure 1C and D).



A

B

C

D

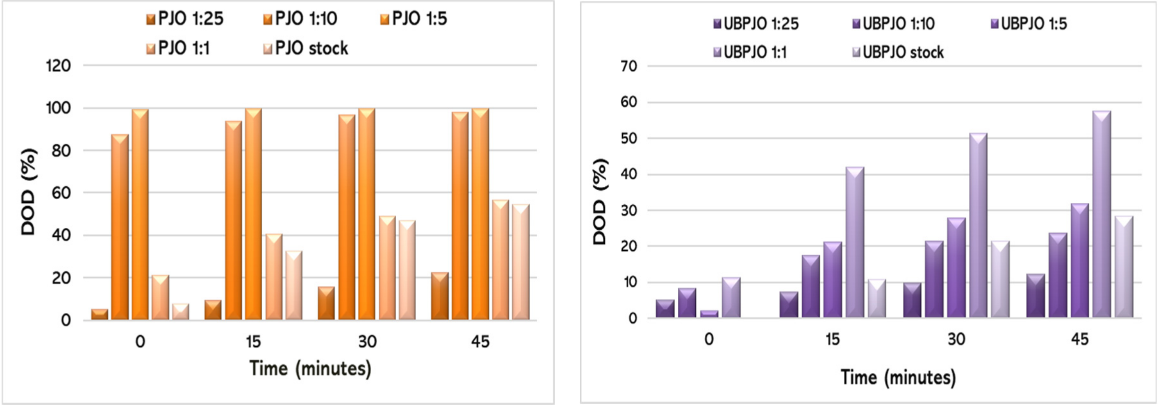
Figure 1. (A,B) Optical density (OD) values of different dilutions of PJO (A) and UBPJO (B); (C,D) Antioxidant capacity of various dilutions of PJO (C) and UBPJO (D) expressed as % decrease of optical density - ΔOD (%); DOD = ΔOD.

2.1.2. ABTS Method

ABTS antioxidant capacity assay was performed at multiple dilutions (1:1, 1:5, 1:10, and 1:25 in ethanol) to evaluate dose-response behavior. We were unable to assess the undiluted samples because an emulsification process rendered them unsuitable for reading at the specified OD. The literature indicates that the complex solubility of oil extracts with multiple components can skew results in ABTS assays (BenchChem Technical Support).

Results from the ABTS method show that the 1:1 dilution of UBPJO has the strongest antioxidant effect among all dilutions, while PJO is highly active at 1:5 and 1:10, consistently with no significant differences. At 45 minutes, the antioxidant effect reached its peak (ΔOD = 99.55% and 97.72%, respectively, Figure 2A). Furthermore, at all dilutions and times, ΔOD for PJO was approximately twice as high as for UBPJO (Figures 2A and B).

For UBPJO, the most pronounced reduction in optical density (ΔOD % at 734 nm) occurred at the 1:1 dilution; higher dilutions of UBPJO (1:5 to 1:25) showed progressively flatter responses (Figure 2B).



A B

Figure 2. Antioxidant effect through the ABTS method, expressed as % decrease of optical density - ΔOD (%) at different dilutions of PJO (A) and UBPJO (B); DOD = ΔOD.

This pattern might indicate potent antioxidant activity, best captured at moderate dilution, where solubility issues in the undiluted oil are resolved without excessive dilution masking the effect.

The ABTS results show an optimal 1:1 dilution for the UBPJO, where maximum radical scavenging (ΔOD = 57.70% at 45 minutes) coincides with greater ethanol solubility (Figure 2B). This highlights the limitations of standard ABTS protocols for oils, as undiluted samples may limit antioxidant (e.g., tocopherols) access to the hydrophilic ABTS$^+$ radical, leading to underestimated activity. The plateau at higher dilutions (>1:5) indicates the assay's linear range is surpassed, confirming strong activity without a proportional dose-response, consistent with reports on oil plant extracts.

Results show that, for undiluted samples, an emulsification process occurred, which prevented accurate measurement of the high concentration of lipophilic compounds. This might be a limitation of the ABTS method in our experiment.

2.3. Sunscreen Properties

The calculated SPF for UBPJO was slightly higher than PJO (32.41 vs. 30.58); the results are shown comparatively with SPF values measured at wavelengths between 290 and 320 nm in Figure 3.

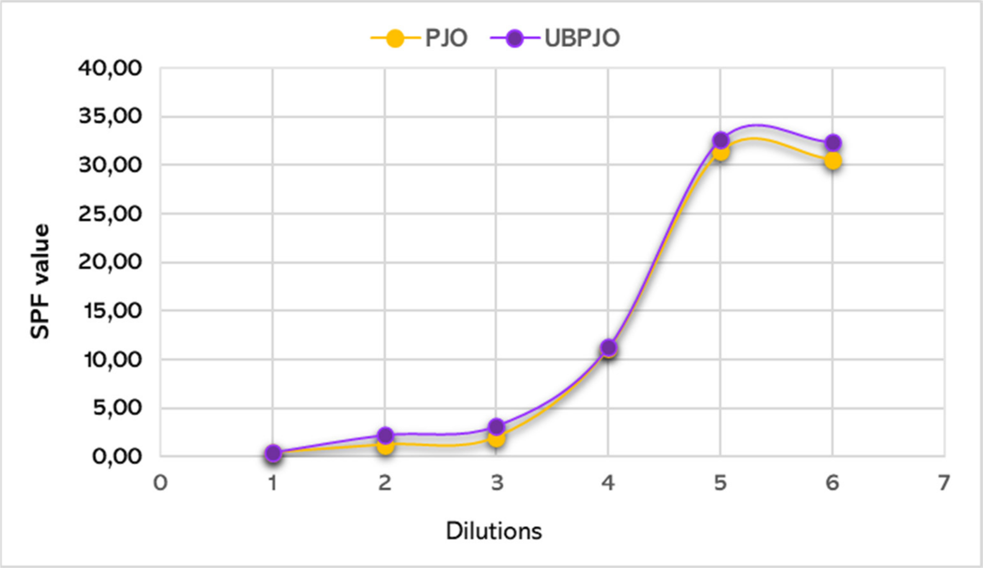


Figure 3. Variation of SPF values, in the wavelength range of 290-320 nm at 5 nm intervals, for PJO and UBPJO; PJO – Jojoba oil with 5% Peppermint Essential Oil; UBPJO – *U. barbata* oil extract. Dilutions 1-6 = 1:10; 1:5; 1:4; 1:2; 1:1; stock solution.

2.3. Antimicrobial Activity

The results of the antimicrobial activity assessment focused on two areas: determining the minimum inhibitory concentration (MIC) and evaluating the ability of PJO and UBPJO to inhibit microbial biofilm formation. To assess oil samples, we used a 20% Tween 80 solution in ethanol for solubilization, allowing the compounds to disperse in the aqueous culture medium. Tween 80 was included as a positive control in the microbiological analyses.

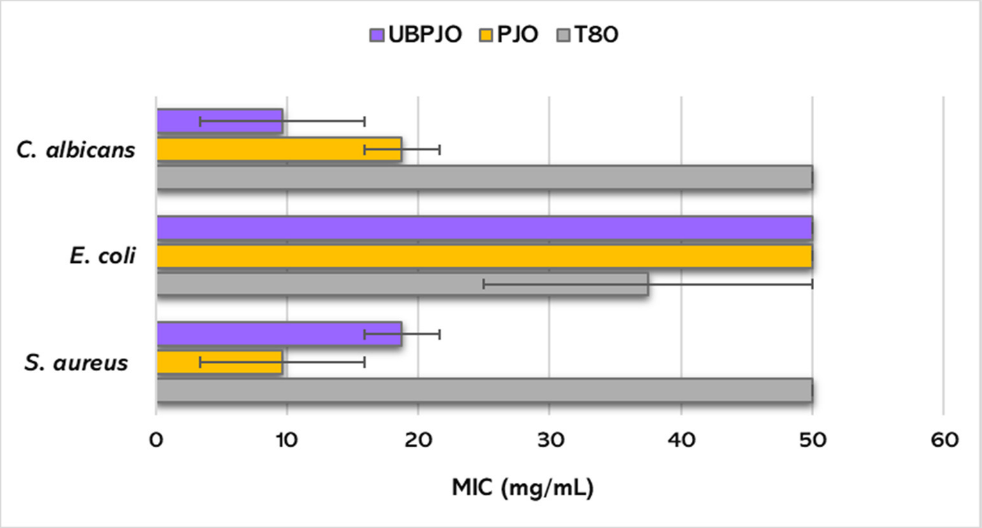
Data from Table 2 and Figure 4A show that UBPJO is significantly more effective than PJO against *S. aureus* and *C. albicans,* as evidenced by lower MIC values ($p < 0.05$). On E. coli, T80 has the highest antifungal effect ($p > 0.05$).

Table 2. Minimum inhibitory concentration (MIC) values.

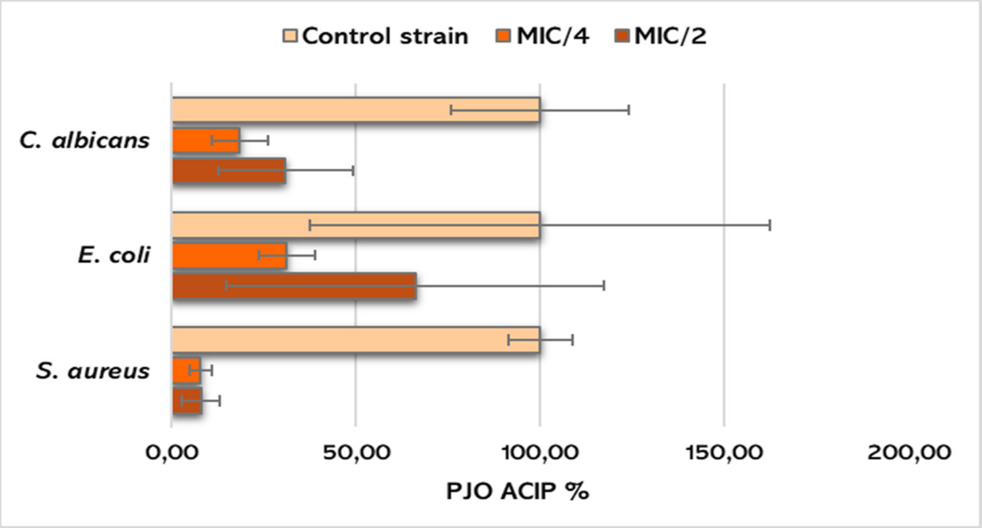
Microbial strain	MIC (mg/mL)		
	Positive control	Oil samples	
	T80	PJO	UBPJO
Staphylococcus aureus ATCC 25923	50 ± 0.00 ^{a}	37.50 ± 0.00 ^{a}	18.75 ± 6.25 ^{a}
Escherichia coli ATCC25922	37.50 ± 12.50	50 ± 0.00	50 ± 0.00
Candida albicans ATCC10231	50 ± 0.00 ^{b}	37.50 ±12.50 ^{c}	9.62 ± 2.87 ^{b,c}

T80 = Tween 80 20% in ethanol; PJO = Jojoba oil enriched with 5% Peppermint oil and 10% vitamin E; UBPJO = *U. barbata* extract in PJO. The differences between values noted with the same superscript letter in the same row are statistically significant.

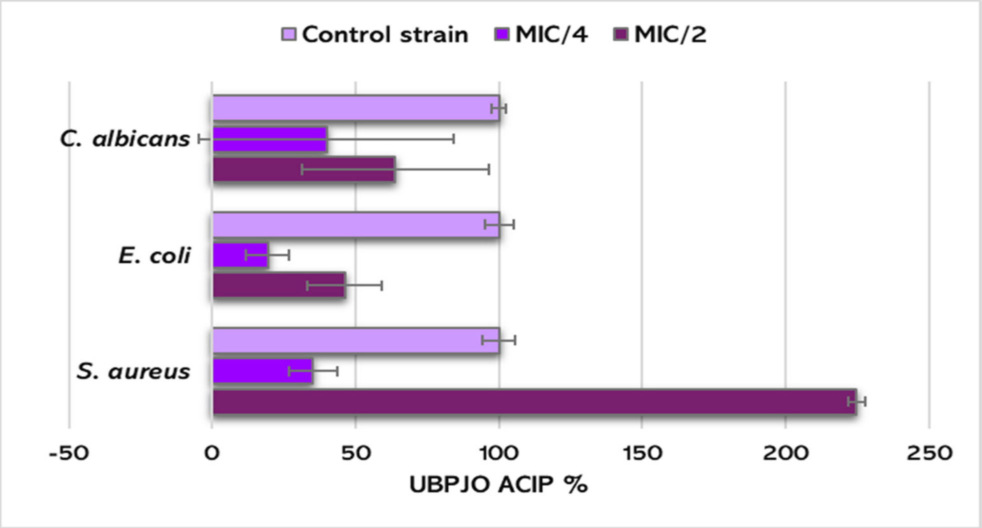
Figures 4B and 4C show the ability of PJO and UBPJO to inhibit biofilm formation by the microbial strains tested at subinhibitory concentrations of MIC/2 and MIC/4.



A



B



C

Figure 4. (A) Antimicrobial activity: MIC values of UBPJO, PJO, and Tween 80 against *C. albicans*, *E coli*, and *S. aureus.* (B,C) Antibiofilm activity: Adherence capacity inhibition percentage (ACIP %) for PJO (B) and UBPJO (C) against tested microorganisms.

PJO has an increased ability to inhibit the adhesion of E coli to the inert substrate than UBPJO at MIC/2 and MIC/4 (66.2 ± 51.18 and 31.34 ± 7.79 vs. 46.17 ± 12.84 and 19.25 ± 7.36, $p > 0.05$, Figure 4B and C).

Conversely, UBPJO at MIC/2 exhibited substantial antibiofilm activity against *S. aureus* (224.63 ± 2.85 vs. 7.95 ± 5.11, $p < 0.001$). Significantly higher activity was exhibited at M/4 (35.01 ± 8.47 vs. 7.87 ± 3.12, $p < 0.01$, Figure 4B and C).

The UBPJO extends the antibiofilm activity to *C. albicans* at MIC/2 more than PJO (63.75 ± 32.66 vs. 30.99 ± 18.42, $p > 0.05$).

3. Discussions

The current study examined a complex oil extract of *U. barbata* in Jojoba oil, enriched with 5% PEO and 10% Vitamin E, for potential skincare uses. The results show synergistic interactions among all active ingredients, yielding beneficial pharmacological properties (antioxidant, photoprotective, and antimicrobial) for this innovative formulation.

Before analyzing the contribution of each component, it is important to recognize that synergy in cosmetic formulations occurs when the combined effect of ingredients exceeds the sum of their individual effects. Each ingredient in this group functions through distinct but complementary biochemical mechanisms, providing multi-layered protection and effectiveness.

Vitamin E serves as the cornerstone of antioxidant synergy in this formulation system, and its contributions are well documented [66]. It donates a hydrogen atom to stabilize free radicals generated by UV exposure, pollution, or oxidative stress [67]. However, in doing so, it becomes a tocopheroxyl radical itself — relatively stable due to its aromatic nature, but no longer active [68,69].

Vitamin E is found in lipid membranes [70,71] and is the first barrier to neutralize free radicals produced by UV radiation [72,73]. During this process, it becomes oxidized and forms a less active radical [74]. Vitamin E primarily functions in the lipid phase, which directly relates to its interaction with jojoba oil, as a lipid-rich carrier.

Topical application of alpha-tocopherol (the active form of vitamin E) has been shown to increase collagen density during the early phase of wound healing, with a significant rise in fibroblast numbers observed in treated animals compared to placebo [75,76]. Vitamin E can also serve as a cofactor to stabilize Vitamin A and protect it from oxidation, suggesting a broader role as a molecular protector of other sensitive actives within a formulation [73,77].

Jojoba oil is not a typical triglyceride oil — its structure closely resembles that of liquid wax, which gives it excellent oxidative stability, high skin compatibility, and greater stability than regular oils [78]. This wax-like structure makes jojoba oil an ideal lipid-phase carrier for fat-soluble ingredients like Vitamin E and components of peppermint oil. The liquid wax structure of jojoba oil stabilizes lipid-soluble actives, such as Vitamin E and terpenoids from peppermint essential oil [79].

The skin compatibility of JO enhances the penetration and bioavailability of co-ingredients [80]. Since Vitamin E functions within the lipid phase, jojoba oil's wax-like lipid matrix provides an ideal environment for Vitamin E to act as an antioxidant at the membrane level. This shows that jojoba oil not only carries Vitamin E but also creates a supportive environment for its activity. It also enhances the synthesis of collagen and hyaluronic acid and diminishes inflammation in human skin [81]

The oxidative resilience of JO decreases the oxidative degradation of sensitive ingredients [82]. Moreover, due to its emollient properties, JO creates a protective film that prolongs the contact time of antimicrobial agents [83,84].

Peppermint oil's antimicrobial properties complement *Usnea barbata's* secondary metabolites activity. While usnic acid and other phenolic metabolites target bacterial cell membranes and metabolic processes, peppermint oil's terpenoids disrupt microbial membrane integrity through a different but complementary pathway, potentially broadening the spectrum of antimicrobial coverage.

Menthol is a well-recognized skin penetration enhancer [85]. By temporarily modifying the lipid structure of the stratum corneum [85–87], peppermint oil can increase the bioavailability of vitamin E in deeper skin layers, enhance the delivery of usnic acid from *U. barbata,* and improve the overall efficacy of the formulation system [88].

Peppermint oil's cooling and anti-inflammatory properties [89] complement Vitamin E's documented anti-inflammatory effects [90]. Studies have shown that Vitamin E in combination with other actives leads to significant decreases in TNF-α expression [91], and peppermint oil's menthol component activates TRPM8 receptors [92], providing an additional, mechanistically distinct anti-inflammatory pathway.

U. barbata contains phenolic secondary metabolites with particular structures; usnic acid is its primary bioactive compound [93].

Usnic acid disrupts bacterial DNA and RNA synthesis and membrane function [94]. When combined with peppermint oil's membrane-disrupting terpenoids [95]. The two agents attack microbial cells through dual mechanisms, potentially reducing the minimum inhibitory concentration (MIC) required for each one — a classic definition of antimicrobial synergy.

Usnic acid possesses its own antioxidant properties through phenolic hydroxyl groups that can scavenge free radicals [96]. This creates a multi-molecular antioxidant network in combination with Vitamin E, resulting in broader radical-scavenging coverage.

Usnic acid has demonstrated UV-absorbing capacity [97], particularly in the UVB range. This complements Vitamin E's photoprotective role (which is antioxidant-based rather than UV-filtering) [98] creating a two-pronged photoprotection system: *U. barbata* absorbs/filters UV photons [99], while vitamin E neutralizes reactive oxygen species generated by UV that pass through [100].

Antioxidant assays such as ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) are commonly employed to evaluate the quality of oil extracts, particularly those rich in lipophilic compounds like tocopherols, carotenoids, and phenolic lipids derived from plants or marine sources. In oil matrices, DPPH often outperforms ABTS due to its superior solubility in non-polar solvents such as ethanol, hexane, or chloroform, enabling direct assessment of antioxidants without extensive emulsification or dilution that could skew results in ABTS assays. This makes DPPH particularly suitable for routine quality control in oil extract manufacturing, where rapid, reproducible measurements of radical-scavenging capacity are prioritized over absolute quantification.

Conversely, ABTS requires the generation of the radical cation (ABTS- $^{+}$) in aqueous or mixed solvent systems, which may pose challenges for oil extracts; poor solubility might lead to an underestimation of lipophilic antioxidants and higher variability.

Research on essential and vegetable oil supplements shows that DPPH provides more consistent results than ABTS, often indicating 1.5-2 times higher capacities but with weaker correlation with in vivo bioactivity because it is sensitive to both hydrophilic and lipophilic substances. Therefore, for oil extracts, DPPH is the preferred method for reliable quality assessment, offering practicality and specificity for lipophilic antioxidants, while ABTS can be used as a complementary tool for broader profiling when solubility issues are addressed [101].

The determination of the sun protection factor (SPF) for vegetable oils used in skincare products is a topic of intense investigation in the literature, highlighting significant variability in results across methodologies, concentrations, and formulation types [102–110]. Sun protection factor (SPF) is a quantitative indicator used in sunscreens to assess a formulation's photoprotective efficacy against ultraviolet (UV) radiation.

In this study, both PJO and UBPJO were subjected to SPF determination by spectrophotometry. The results obtained indicated SPF values of ~30.58 for PJO and ~32.41 for UBPJO. These values demonstrate the ability of both formulations to act as natural photoprotective agents, and the smaller difference between the two confirms an increase in efficiency when using the combination with lichen extract.

In both oil samples, PJO and UBPJO, a progressive increase in absorbance towards lower wavenumbers (closer to 290 nm) was observed. This aligns with the literature, which indicates that the increased absorbance near 290 nm results from the higher-energy excitation of the conjugated π-electron system, a common absorption characteristic of phenols and other aromatic compounds, and matches the compositions of both samples determined in previous research. However, UBPJO shows slightly higher absorbance at all measured wavelengths than PJO, and this is due mainly to the synergistic interaction between the complex oil base's bioactive compounds and the phenolic lichen secondary metabolites [111,112], including usnic acid [113]. *U. barbata* constituents ensure a higher concentration of chromophoric groups capable of absorbing UVB radiation. In addition to absorbing UV light directly, these phenolic compounds also have antioxidant properties. The dual function of these metabolites helps enhance photoprotection through two mechanisms:

- The absorption of UV photons is known as primary photoprotection. The energy is then dissipated through non-radioactive mechanisms [114].
- The active ROS (reactive oxygen species) neutralization is generated by UV exposure. This secondary photoprotection is also enhanced by the presence of alpha-tocopherol (the form of vitamin E) [115,116].

Pure vegetable oils generally exhibit low intrinsic SPF values, but they have recently been widely used in sunscreen formulations due to their unique UV-absorption properties and improvements in the spreadability and skin-adsorption characteristics of the final products [117,118]. The literature reports SPF values of 6.02 for JO, 9.28 for olive oil, 18.81 for carrot seed oil, and 22.04 for wheat germ oil [119] The SPF values of natural Jojoba oil reported in the literature are generally similar to those of other vegetable oils [120].

Montenegro et al. studied the use of 1% pomegranate and shea oil as natural UV filters to improve the SPF of sunscreen preparations [118]. Another recent study reported that the SPF of a cream base + Oksibenzon 2% + Octil metoximate 5% was significantly increased from 21.12 to 37.01 by adding 10% JO.

The SPF value of 30.58 for the PJO is consistent with literature data for vegetable oils combined with other compounds (PEO and vitamin E, as used in our research). The measured absorbance in the 290-330 nm spectral range can be attributed to the optical properties of the JO and PEO. Jojoba oil contributes to moderate UV absorption and, consequently, to a slight photoprotective activity, while PEO and vitamin E contribute to minor UV absorption and antioxidant activity.

Furthermore, the SPF value of 32.41 for UBPJO indicates that the *U. barbata* extract can moderately enhance the photoprotective performance and UV-absorbing capacity of formulations when they are incorporated into lipid-based matrices. Several studies have reported that usnic acid and other phenolic secondary metabolites of *U. barbata* exhibit antioxidant, anti-inflammatory, antiviral, and antimicrobial effects. These biological activities are particularly relevant for photoprotection because UV radiation induces oxidative stress and an anti-inflammatory skin response [99,121].

The antimicrobial activity was evaluated against the most common pathogens (*S. aureus, E. coli,* and *C. albicans*); our results revealed a significant antimicrobial and antibiofilm activity of UBPJO on *S. aureus* and *C. albicans.* The antimicrobial potential of UBPJO is mainly due to the synergistic action of its 2 ingredients, *U. barbata* and PEO.

U. barbata efficacy against *S. aureus* is mainly due to phenolic secondary metabolites, especially usnic acid [33,34]. Usnic acid's anti-staphylococcal activity involves a multitarget approach. It primarily disrupts the bacterial cell membrane, rapidly inhibits RNA, DNA, and protein synthesis, and impairs peptidoglycan/fatty acid biosynthesis [122]. Moreover, usnic acid can inhibit multidrug resistance (MDR) efflux pumps, reducing MRSA's ability to expel antibiotics and thereby increasing its sensitivity to drugs such as vancomycin and norfloxacin. disrupts pre-formed biofilms and inhibits the formation of new ones by reducing surface attachment and inhibiting bacterial quorum sensing [122,123].

Our previous research reported a strong antibacterial and antibiofilm activity of Peppermint essential oil from Fares S.A. against *S. aureus* [124]. The results align with those of other studies from the scientific literature [125,126]. Thus, PEO demonstrates significant potential as an antibacterial and anti-biofilm agent against *S. aureus.* PEO appears to cause irreversible damage to the cell membranes, as evidenced by increased membrane permeability and leakage of nucleic acids, proteins, and ATP. Additionally, PEO can inactivate mature *S. aureus* biofilms, highlighting its promising role in managing bacterial infections and biofilm-related challenges [46]. UBPJO is rich in pulegone (41.66%) and isomenthone (19.50%); both are very effective against *S. aureus* and act through a common mechanism of terpenoids [127–129].

Jojoba oil could also contribute to the inhibitory activity against *S. aureus,* according to Al-Ghamdi et al. [130].

According to the previously published studies [131–134], the key mechanisms of action of usnic acid against *C. albicans* include the following:

- Anti-biofilm activity: usnic acid reduces the biomass and thickness of mature biofilms, reducing metabolic activity in sessile cells.
- Inhibition of morphological transition: It restricts the transformation from yeast to hyphae, a critical stage in *C. albicans* infection and virulence.
- Oxidative/nitrosative stress: usnic acid induces both intracellular and extracellular reactive oxygen species, leading to cell damage.

- Reduced exopolysaccharide layer (EPS): It lowers the sugar content within the biofilm's protective exopolysaccharide layer.
- Targeting drug-resistant strains: It shows efficacy against azole-resistant C. *albicans.*

The lipophilic characteristics of PEO, particularly its primary component menthol, facilitate its integration into the fungal phospholipid bilayer. This interaction induces significant alterations in membrane fluidity and permeability, leading to the leakage of essential intracellular constituents, including calcium, potassium, and magnesium ions; proteins; and nucleic acids. Consequently, these alterations lead to cellular death. Peppermint oil effectively diminishes the ergosterol content in fungal cell membranes. Ergosterol is a fundamental component of membrane stability; thus, its reduction undermines membrane integrity [135–138]. PEO has demonstrated the capability to completely inhibit C. albicans biofilm formation, thereby preventing the growth and survival of these pathogenic yeast strains [136]. PEO has been shown to stimulate ROS production, leading to oxidative damage to various cellular components, underscoring the importance of understanding its effects on cell health. Elevated ROS levels can damage DNA, leading to fragmentation. This process can trigger apoptosis-like cell death in fungi such as *C. albicans,* underscoring the potential implications of peppermint oil for antifungal applications [135,137]. Moreover, PEO can interfere with mitochondrial energy metabolism by inhibiting respiratory enzymes, thereby reducing ATP production in fungal cells [133,139,140].

4. Materials and Methods

4.1. Materials

Jojoba oil (JO) obtained by cold pressing *Simmondsia chinensis* seeds and Vitamin E were supplied by Fagron Hellas (Trikala, Greece). Jojoba oil is highly pure and suitable for cosmetic applications. Its liquid wax composition, primarily long-chain esters, provides oxidative stability, a non-greasy texture, and compatibility with human skin. In our experiment, it was combined with 10% Vitamin E.

Peppermint essential oil (PEO) was purchased from Fares S.A. Orastie; its CG-MS analysis was previously reported [58]. It was diluted in JO (carrier oil) mixed with 10% Vitamin E to 5% concentration (PJO).

U. barbata lichen was harvested in March 2024 from the Călimani Mountains, Romania (47°28′ N, 25°13′ E, at an altitude of 900 m). The freshly collected lichen thalli were separated from impurities, then dried at 18–25 °C in an herbal room, protected from sunlight. Dried lichen preservation for an extended period was performed in similar conditions. It was identified by the Department of Pharmaceutical Botany of the Faculty of Pharmacy at Carol Davila University of Medicine and Pharmacy using standard methods. A voucher specimen is maintained in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Carol Davila University of Medicine and Pharmacy (UBL 3/2024, Ph-UMFCD) [29].

All chemicals, solvents, and reagents were of analytical grade.

4.2. Preparation of U. barbata Oil Extract

The oil extract was prepared by cold maceration, which preserves the integrity of bioactive compounds and prevents thermal degradation. The harvested lichen samples were ground and passed through successive 2.5 mm sieves (DIN 1171) and 1.2 mm mesh (DIN 117) for homogenization. Almost 20 g of this mass was accurately weighed using a Kern analytical balance, placed in a 1000 mL brown glass container, and 500 mL of JO enriched with 5% PEO and 10% Vitamin E was added. The sample was macerated for 3 months in a light-protected location at a constant temperature (21-22 °C) [41].

The brown container with both components was manually shaken daily for three months; after this period, the oil extract (UBPJO) was filtered into a brown vessel with a sealed plug and preserved in a plant room, sheltered from sunlight [41].

4.3. Antioxidant Activity

4.3.1. DPPH Method

We tested the radical-scavenging capacity of the UBPJO using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, as previously described [141–143]. The DPPH method is based on the discoloration of a solution containing the DPPH free radical (Sigma-Aldrich, USA) upon interaction with antioxidants present in the tested sample. For each sample, several dilutions in methanol were prepared (1:1, 1:5, 1:10, and 1:25) to assess the dose dependency of the antioxidant effect.

Each sample was used at a 1:2 ratio with the DPPH working solution, which had an initial OD of approximately 0.8.

We performed a kinetic assessment, recording the decrease in OD at 517 nm over 1 hour. Also, we conducted endpoint measurements, after an incubation period of 5, 30, and 60 min respectively, analyzing the decrease of OD:

$$\Delta OD\ (\%) = 100 \times (OD_{\text{DPPH + sample}} - OD_{\text{sample}})/OD_{\text{DPPH}}.$$

This was considered to express the antioxidant effect of the tested oil samples.

4.3.2. ABTS Method

We used an ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))-based method to assess the antioxidant capacity of the extract [141]. This method is based on measuring the sample's scavenging capacity of the ABTS+· free radical. The stock reagent containing ABTS 7mM and $K_2S_2O_8$ 2.45mM was kept in the dark for 16 h at 4 °C for the generation of the green ABTS+· free radical. The working solution (WS) was obtained by diluting the stock with distilled water until an appropriate OD (0.8) was reached at 714 nm.

Samples (undiluted as well as 1:1, 1:5, 1:10, and 1:25 dilutions) were incubated with ABTS WS in a 1:3 ratio, and OD was measured at 714 nm after an incubation period of 10 min. A blank was prepared using ethanol instead of the sample. Results are presented as the % decrease of optical density (ΔOD):

$$\Delta OD\ (\%) = 100 \times (OD_{\text{blank}} - OD_{\text{sample}})/OD_{\text{blank}}.$$

These are directly correlated to the antioxidant capacity of the samples.

4.4. Sunscreen Properties

1 mL of each oil sample was added to a 100 mL volumetric flask and diluted with ethanol to the mark. Further, it was ultrasonicated for 5 min. The sun protection factor (SPF) of the oil samples was determined using a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer (Perkin-Elmer Inc., Waltham, MA, USA) in transmission mode. Oil samples were placed in microcuvettes with a 10 mm light path, and absorbance spectra were recorded over 290-320 nm at 5 nm intervals, with three determinations at each point. SPF values were calculated according to established protocols using the Mansur formula [144–152] (Eq 1).

$$SPF = CF \times \sum_{320}^{290} EE_{(\lambda)} \times I_{(\lambda)} \times abs_{(\lambda)} \tag{1}$$

where CF is the correction factor (usually, CF=10), EE(λ) is the erythemogenic effect of radiation at wavelength λ, I(λ) is the intensity of solar light at wavelength λ, and abs (λ) is the absorbance of wavelength λ by the preparation solution.

4.5. Antimicrobial Activity

To evaluate the antimicrobial activity of PJO and UBPJO, we determined the minimum inhibitory concentration (MIC) and the adhesion capacity of the strains to an inert substrate (PICA% = percentage inhibition of adhesion capacity), using the following reference strains purchased from American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC25922, and *Candida albicans* ATCC10231.

4.5.1. Determination of Minimum Inhibitory Concentrations

Suspensions with an optical density of 0.5 McFarland for bacteria and 1 McFarland for fungi were prepared, and the following were distributed in the 96-well plates: 100 µL of TSA (Tryptic Soy Agar, Sigma-Aldrich Merck (Dartmand, Germany) liquid culture medium (for bacteria) and RPMI 1640 (American Biorganics, Buffalo, NY, USA) (for fungi), 100 µL of a 100 mg/ml solution of the substances investigated in the first well, after which a binary serial dilution scheme was performed up to well 10, and 20 µL of suspension from the strains to be analyzed, from well 1 to well 11 (11 representing the positive growth control, and 12 representing the negative control). The samples were processed in duplicate. The absorbance readings were performed at 620 nm using a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) [153].

4.5.2. Evaluation of the Influence of Oil Samples on Microbial Adherence Capacity to the Inert Substratum

The influence of UBPJO and PJO on biofilm formation is evaluated using the previously described method to determine MIC values. After reading these values, the degree of adhesion to the inert substrate of the tested strains is evaluated by following these steps: fixation with methanol, staining with 1% crystal violet solution resuspended in 33% acetic acid.

The percentage of capacity of adherence inhibition (%) was determined using the following formula:

$$\text{ACIP (\%)} = (A_s - A_{blank}) \times 100/(A_c - A_{blank})$$

where A_s is the absorbance at 490 nm of the tested samples, and A_c is the absorbance at 490 nm of the control [153].

4.6. Data Analysis

Almost all measurements were performed in triplicate to ensure reproducibility, and the results are expressed as mean ± standard deviation. Data analysis was performed using XLSTAT Premium v.2025.2.0.1232 (Lumivero, Denver, CO, USA) and Microsoft Excel v. 16.0 19328 (Microsoft Corporation, Redmond, WA, USA). ANOVA single factor was used to detect significant differences between variables ($p < 0.05$) [124].

5. Conclusions

The present study investigated the antioxidant, photoprotective, and antimicrobial properties of a complex oil lichen extract, *U. barbata* in Jojoba oil enriched with 5% Peppermint essential oil and 10% Vitamin E, with potential applications in the cosmetic field. The results suggest synergy between the active ingredients across all evaluated pharmacological activities. UBPJO offers promising perspectives for the further development of multifunctional formulations that could protect the skin against oxidative stress and UV radiation, maintain hydration, and support skin health through antioxidant and antimicrobial properties.

In the future, advanced studies may focus on incorporating UBPJO into various cosmetic formulations to investigate its properties and valorize its benefits in a skincare routine or in the therapy of various skin conditions.

Author Contributions: Conceptualization, M.A.D., E.A.O., V.P., and D.L.; methodology, M.A.D., M.I.N., E.A.O., A.U., A.R.U., V.P., A.M.M., V.B., A.L.A. and D.M.; software, V.P.; validation, E.A.O., A.M.M., A.L.A., D.L., and D.M.; formal analysis, V.P.; investigation, M.A.D., M.I.N., E.A.O., A.U., A.R.U., V.P., A.M.M., V.B., R.G.C., A.L.A., D.L. and D.M.; resources, M.A.D., M.I.N., E.A.O., A.U., A.R.U., V.P., A.M.M., V.B., R.G.C., A.L.A., D.L. and D.M.; data curation, M.A.D., M.I.N., E.A.O., A.U., A.R.U., V.P., A.M.M., V.B., R.G.C., A.L.A., D.L. and D.M.; writing—original draft preparation, M.A.D., M.I.N., E.A.O., A.U., A.R.U., V.P., A.M.M., V.B., R.G.C., A.L.A., D.L. and D.M.; writing—review and editing, E.A.O., V.P., A.M.M., and D.M.; visualization, M.A.D., M.I.N.,

E.A.O., A.U., A.R.U., V.P., A.M.M., V.B., R.G.C., A.L.A., D.L. and D.M.; supervision, D.L.; project administration, D.L.; funding acquisition, E.A.O. and R.G.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the manuscript text.

Conflicts of Interest: The authors declare no conflicts of interest.

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