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Endolichenic Fungi Extracts: A Promising Alternative for Ultraviolet Protection in Cosmetics?

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Synopsis

Lichen-derived extracts are a potential source for skin health products due to their antioxidant activity that can prevent damage caused by ultraviolet (UV) rays. The slow natural growth and limited reproduction of lichens limit their use. However, endolichenic fungi (ELF) from lichen thalli can serve as a more efficient, rapid, and standardized biological resource for the cosmetic industry. We aimed to investigate the UV protection of *Lobaria pulmonaria*, *Bryoria capillaris*, and *Usnea* sp., and isolated ELF extracts. Total antioxidant, phenolic-flavonoid content, sun protection factor (SPF), and tyrosinase inhibitory activities of lichen and ELF extracts were also investigated. As a result, ELF extracts were found to have antioxidant content close to, and in some cases, much higher than lichen species. While SPF values of lichen species varied between 31.45–31.80, results close to lichens were found in the range of 6.54–32.01 values of ELF extracts. Interestingly, lichen extracts did not have tyrosinase inhibitory activity, while some ELF extracts had tyrosinase inhibitory activity in the range of 2.49–38.44. The results of this study suggest that ELF extracts may have the potential to be used as innovative UV protectants in the cosmetic industry.

INTRODUCTION

Lasting exposure to harmful solar ultraviolet (UV-C (100–280 nm), UV-B (280–320 nm), and UV-A (320–400nm)) radiation causes chronic or acute skin damage such as skin cancer, photoaging, wrinkles, and sunburn.[1–3] As a result of photoaging, thickening of the skin, wrinkles, and chronological damage known as pigmentation occur. In this case, excessive production of intracellular reactive oxygen species (ROS) is known to cause photoaging, excessive melanin production, DNA damage, and skin cancer. The mechanism of skin pigmentation protects the skin from UV damage. Melanin, which causes skin pigmentation, also protects our skin from UV damage by absorbing UV rays.[4,5] However, excessive melanin production is closely related to problems such as solar lentigo and freckle formation.[6,7] Therefore, tyrosinase inhibitors are being investigated for application in cosmetic products for skin lightening and depigmentation. There are many known tyrosinase inhibitors such as hydroquinone, arbutin, and kojic acid. However, most of them are reported to have low

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activity or to have negative effects such as mutagenicity and cytotoxicity.[8,9] Therefore, there has been a recent need to identify effective and safer tyrosinase inhibitors from natural sources, especially for the cosmetic industry.[10]

Since it is known that UV increases ROS and that antioxidants prevent the damage caused by ROS, antioxidants have recently come to the fore in the cosmetic industry. Antioxidants play an active role in preventing, repairing, and reducing cellular damage caused by free radicals.[11] Recently, there is increased focus on taking precautions against photoaging and pigmentation problems caused by UV damage. UV radiation is emphasized as one of the main causes of photoaging and structural changes in the skin, and other forms of solar radiation, including visible and infrared light, may also play a role in photoaging.[12] UV radiation increases the risk of long-term damage such as photoimmunosuppression and photocarcinogenesis as well as photoaging.[13] There has also been an increase in UV-induced melanoma cases in various countries in recent years, and many health authorities are working intensively on increasing the awareness of this issue and increasing the use of sunscreen products.[14,15] Therefore, the discovery of natural products with high antioxidant activity is believed to be important.

The need for cosmetic products is increasing every year all over the world. While the world cosmetics market had a market share of US $380.2 billion in 2019, it is expected to reach US $463.5 billion with a compound annual growth rate of 5.3% (CAGR) from 2021 to 2027.[16] Sun care products are especially among the products with the fastest growth potential in the cosmetics market. The market for these products was valued at US $11.4 billion in 2021 and is expected to increase to US $17.6 billion by 2027 with a 7.3% CAGR over 2022 to 2027.[17] One of the main reasons for this growth is the increased awareness of the harmful effects of UV rays. Sunscreens are widely used in daily life due to their functions such as filtering, reflecting, and dispersing UV rays.[18] In particular, consumers' preference for organic-based products and the development of innovative and advanced sun care products also contribute to the growth of this market.

Natural products have been the cornerstone of cosmetics and pharmaceutical production in many parts of the world for years. Recently, there has been an increasing interest in cosmetic products containing natural herbal extracts, especially for components such as pigment removers and sunscreens, leading to a growing demand for natural extracts in the global cosmetics market. Due to the increasing demand of consumers for healthy and environmentally friendly products, the use of natural herbal ingredients has increased in the cosmetics sector.[19]

Lichens are symbiotic associations of fungi (mycobionts), green algae and/or cyanobacteria (photobionts). In addition to the fungal partner in this mutualism, lichens are also associated with endolichenic fungi that reside within their thalli.[20] Lichens producuse nearly 1,050 specific secondary metabolites that have antioxidant and antimicrobial effects. Despite the unique biological activity of lichens, their slow growth rate, and limited numbers in nature make their application in various industries difficult.[21] There are also difficulties in the *in vitro* production of lichens. However, ELFs are fungal species that live in the lichen thallus and are easy to grow in lab scale in a shorter time and in large quantities. Therefore, the importance of producing ELF with similar bioactivities that are not being used in industries has emerged. ELF are considered promising biological resources for various fields, given their ability to produce many valuable bioactive metabolites.[22] Studies on the bioactivities of ELF isolated from lichens are quite limited. Identifying and evaluating

the composition and efficacy of ELF is particularly important in industries such as pharmaceuticals and cosmetics. It is expected that the large-scale production of ELF, whose bioactivity has been determined, will be used in various sectors, ensuring sustainability, creating environmentally friendly products, and contributing economically.

In particular, consumers prefer sunscreen products with natural ingredients to take precautions against various skin problems that may occur as a result of exposure to UV rays. For this purpose, it was aimed to determine the *in vitro* cosmetic activities of ELF extracts isolated from lichen thalli and to determine their potential use as a potential source in the cosmetic industry. First, ELF isolation was performed from *Usnea* sp., *Lobaria pulmonaria*, and *Bryoria capillaris* to determine *in vitro* the potential efficacy of cosmetic potentials of lichens and isolated ELF extracts. Total antioxidant activities, total phenolic and flavonoid contents, SPFs, and tyrosinase inhibitory activities were determined.

MATERIALS AND METHODS

COLLECTION LICHEN SAMPLES AND ISOLATION OF THE ENDOLICHENIC FUNGAL SPECIES

The lichen samples of *Usnea* sp., *Lobari pulmonaria*, and *Bryoria capillaris* were collected from the Aladağ region of Bursa.

For the isolation of endolichenic fungi, surface sterilization of *Usnea* sp., *L. pulmonaria* and *B. capillaris* lichen samples was performed.[23] For surface sterilization of lichen samples, they were treated sequentially with 95% ethanol (1 minute), 10% sodium hypochlorite (3 minutes), and 70% ethanol (1 minute) after washing with water, and then dried on a sterile filter paper. Sterile lichen samples were then cut into small 1x1 cm pieces and plated on potato dextrose agar. The plates were incubated at room temperature for 14 days. After the incubation period, different numbers of ELF samples of each lichen species were purified and pure ELF cultures were obtained.

The isolated and purified samples of ELF were labeled with the following codes: T04-P01, T04-P03, and T04-P13 for *Lobari pulmonaria*; T20-B02, T20-P07, T20-P10, T20-P26, and T20-P27 for *Bryoria capillaris*; and T22-P07 and T22-B07 for *Usnea* sp.

EXTRACTION OF LICHEN AND ELF SAMPLES

For extraction from lichen samples, they were first washed and dried. Lichen samples were then placed in sterile bottles with acetone and left in the dark for 24 hours. After 24 hours, the samples were filtered through filter paper. Acetone was removed from the lichen extracts using a rotary evaporator, and 1 mg of the lichen extracts was weighed and dissolved in 1 mL of dimethyl sulfoxide (DMSO), adjusted to a concentration of 1 mg/mL and stored at +4°C. Pure ELF isolates were incubated at 25°C for 21 days in 250 mL Erlenmeyer flasks containing 150 mL of potato dextrose broth. Each fungal culture was filtered to separate the filtrate from the molds. After filtration, an equal amount of ethyl acetate was added to create an organic phase. The organic phase was then evaporated to obtain dry extracts and the extracts were adjusted at a concentration of 1 mg/mL DMSO and which were stored at +4°C.

ANALYSIS OF TOTAL ANTIOXIDANT, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT

The total antioxidant activity of lichen and ELF extracts (1 mg/mL) was determined using the 96-well plate methods.[24] First, 100 μL of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol (40 μg/mL) and 50 μL of 1 mg/mL extract in DMSO were added to a 96-well plate. The mixture was incubated in the dark for 30 minutes. After incubation, absorbance was measured at 517 nm against the blank using a Cytation-3 microplate reader. L-Ascorbic acid (1 mg/mL) was used as a positive control. The percentage of radical scavenging activity was calculated using the following formula:

$$\%\text{DPPH scavening activity} = \left[(A_C - A_S)/A_C\right] \times 100$$

- A_C: Absorbance of control
- A_S: Absorbance of sample

Total phenolic content (TPC) of lichen and ELF extracts (1 mg/mL). Briefly, 40 μL of lichen and ELF extracts, 100 μL of Folin-Ciocalteu reagent and 75 μL of 7.5% Na2CO3 were added to each well and incubated. After incubation, absorbance was measured at 750 nm versus blank using a Cytation-3 microplate reader. Samples were measured in triplicate and a standard curve was constructed using gallic acid starting at a concentration of 1 mg/mL. Phenolic content was expressed as gallic acid equivalents.[25]

The total flavonoid (TFC) content was determined according to the method of Yang *et al.*[26] 75 μL of sample solutions (1 mg/ml) and 75 μL of 2% $AlCl_3$ were mixed in a 96-well plate. After 15 minutes of incubation, the absorbance was measured against the blank at 435 nm using a Cytation-3 device. A standard graph was prepared from a stock solution of rutin at a concentration of 1 mg/mL. The flavonoid content was expressed as rutin equivalents.

IN VITRO DETERMINATION OF SPF

In vitro SPF values of lichen and ELF extracts were calculated according to the method of Mansur *et al.*[27] A measure of 1 mg of each extract was dissolved in 1 mL of methanol and analyzed by UV spectrophotometry from 290 to 320 nm. Methanol was used as the blank. Rutin was used as a positive control. After the measurements, SPF values were calculated according to the following formula:

$$\text{SPF} = \text{CF} \times \Sigma 320 - 290 \times \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{ABS}(\lambda)$$

- CF = 10 (correction factor)
- EE(λ) = erythematogenic effect
- I(λ) = sun intensity
- ABS(λ) = absorbance

TYROSINASE INHIBITORY ACTIVITY ASSAY

In the tyrosinase inhibition activity, tyrosinase enzyme was used as the enzyme and l-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate.[28] Lichen and ELF extracts were prepared homogeneously in DMSO at 1 mg/mL. To the wells on the microplate, 150 μL of phosphate buffer (0.05 M, pH = 6.8), 10 μL of 1 mg/mL extracts, and 20 μL of enzyme solution were added. The microplate was shaken for 3 minutes in a Cytation-3 device, and

the initial absorbance was read at 475 nm. This solution was incubated for 10 minutes at 37°C. After 10 minutes, 20 μL of the substrate (8.5 mM L-DOPA) was added. The mixture was incubated for another 10 minutes at 37°C, and the final absorbance was read at 475 nm. 1mg/mL kojic acid was used as a positive control.

The percent inhibition of tyrosinase enzyme was calculated using the equation:

$$\%\ \text{inhibition} = (A_{\text{control}} - A_{\text{sample}}) \div A_{\text{control}} \times 100$$

RESULTS AND DISCUSSION

Continuous exposure of human skin to UV radiation from the sun leads to the formation of ROS. The presence of antioxidants and phenolic compounds in cosmetic products are very important in preventing the ROS mechanism. In order to get rid of the harmful effects of UV radiation, natural products with defined bioactivity have been frequently preferred in the cosmetic industry in recent years. Consumers have also become aware of the environmental and human health toxicity of synthetic materials used in cosmetic products, and prefer products with natural ingredients.[29,30] In this study, *Usnea* sp., *L. pulmonaria*, and *B. capillaris* lichens and their ELF extracts were tested in terms of antioxidant activity, total phenolic-flavonoid contents, SPF values and tyrosinase inhibitory activity at a concentration of 1 mg/mL and the results were shared.

ISOLATION OF THE ENDOLICHENIC FUNGAL SPECIES

Different endolichenic fungi were isolated from each of *Usnea* sp., *L. pulmonaria,* and *B. capillaris* lichens and purified. Three different ELF were isolated from *L. pulmonaria* lichen, five from *B.capillaris* lichen and two different ELF from *Usnea* sp. lichen. Extracts of each ELF species were used in the experiments (Table I).

TOTAL ANTIOXIDANT, TOTAL PHENOLIC, AND TOTAL FLAVONOID CONTENT

Lichens are used by the public in the food, textile, cosmetics, perfumery, and medicine sectors thanks to their various bioactivities. They are especially prevelant in the cosmetic industry, where lichen extracts are used in face/body lotions and baby creams.[31,32] The antioxidant,

Table I
Extracts of ELF Species Used in the Experiments

L. pulmonaria	*B. capillaris*	*Usnea* sp
T04-P01	T20-B02	T22-P07
T04-P03	T20-P07	T22-B07
T04-P13	T20-P10	
	T20-P26	
	T20-P27	

In this study, ELF was isolated from three different lichen species: *L. pulmonaria*, including T04-P01, T04-P03, and T04-P13; *B. capillaris*, including T20-B02, T20-P07, T20-P10, T20-P26, and T20-P27; *Usnea* sp., including T22-P07 and T22-B07.

phenolic, and flavonoid contents of lichens have been proven by various studies.[33,34] However, the small amount of lichens in nature limits the use of these ingredients in many sectors, including cosmetics. Therefore, it is important to detect and prove the antioxidant and bioactivities of ELF species isolated from lichens, especially in order to prevent skin damage caused by UV. The reason for this is the slow growth of lichens in nature and their limited availability, which limits their use. However, endolichenic fungi isolated from lichens are thought to be important for their use in the cosmetic industry as they grow faster and their extracts are obtained in a standardized manner.[35] For these reasons, total antioxidant, phenolic and flavonoid contents of lichens, and isolated ELF extracts were determined in this study.

The total antioxidant activity of extracts from *Usnea* sp., *L. pulmonaria,* and *B. capillaris* lichens and their ELF extracts was determined using the DPPH method *in vitro*. In this study, the total antioxidant activities of lichen extracts at 1 mg/mL concentration were found to be 34.86% for *B. Capillaris*, 21.74% for *L. Pulmonaria*, and 23.94% for *Usnea* sp. extracts. ELF extracts isolated from *L. pulmonaria* lichen exhibited similar antioxidant activities as their host lichen extracts as shown in Table II (18.82–24.01). Among all extracts, the highest DPPH activity was determined for T20-P26 (91.77%) and T20-B02 (87.82%) isolated from *B. capillaris* lichen. T22-P07 ELF extract isolated from *Usnea* sp. exhibited 70.23%, and T22-B07 extract exhibited 74.26% total antioxidant activity, which was considerably higher than that of the *Usnea* sp. from which they were isolated. It was observed that T20-P26, T20-B02 isolated from *Bryoria* and T22-P07, T22-B07 isolated from *Usnea* sp. were comparable to and even higher than the positive control L-ascorbic acid at a concentration of 1 mg/mL. As a result, when the total antioxidant activities were examined, it was observed that especially T20-B02, T20-P26 isolated from *B.capillaris* and T22-P07, T22-B07 ELF extracts isolated from *Usnea* sp. had higher antioxidant activities than their host lichen species. It is known that antioxidants play a role in preventing damage

Table II
Antioxidant Activity of Lichen and ELF Extracts

Name of the lichen and ELF	DPPH	TPC (mg GAE/g extract)	TFC (mg Rutin/g extract)
Positive control	L-Ascorbic Acid (1 mg/ml) 74.20	Gallic Acid (1 mg/ml) 444.67	Rutin (1 mg/mL) 1008.96
Lobaria pulmonaria	21.74 ± 0.002	86.53 ± 13.39	ND
T04-P01	18.82 ± 0.003	8.85 ± 0.33	399.33 ± 2.31
T04-P03	22.57 ± 0.003	16.10 ± 9.31	212.37 ± 1.28
T04-P13	24.01 ± 0.02	23.42 ± 0.71	204.75 ± 1.15
Bryoria capillaris	34,86 ± 0.037	35.57 ± 2.433	ND
T20-B02	87.82 ± 0.01	128.87 ± 6.59	ND
T20-P07	11,89 ± 0.008	20.73 ± 5.90	274.27 ± 2.02
T20-P10	18.82 ± 0.01	16.81 ± 6.52	373.93 ± 3.89
T20-P26	91.77 ± 0.001	129.37 ± 10.58	51.45 ± 0.85
T20-P27	27.21 ± 0.003	33.03 ± 17.45	ND
Usnea sp.	23.94 ± 0.003	83.14 ± 5.57	263.632 ± 1.63
T22-P07	70.23 ± 0.002	77.68 ± 12.02	80.163 ± 1.02
T22-B07	74.26 ± 0.01	96.08 ± 6.04	ND

ND: No data

such as sun-induced pigmentation and anti-aging.[36,37] Although lichens are known to be a potential source of antioxidants, their slow growth and limited numbers restrict their use in the cosmetics industry. From this point of view, the determination of high antioxidant content in ELF extracts in our study shows that it can be evaluated as a potential antioxidant source in cosmetic products and can be used as a sustainable and environmentally friendly source with economic benefits.

The TPC was calculated in terms of gallic acid equivalent. Although *L. pulmonaria* had the highest phenolic content among the lichen species (86.53 mg GAE/g), the phenolic contents of ELF extracts isolated from this lichen were found to be quite low. On the other hand, T20-B02 and T20-P26 isolated from *B. capillaris* had the highest phenolic content, with 128.87 mg GAE/g and 129.37 mg GAE/g, respectively, similarly to these extract total antioxidant activities. The TPC of *Usnea* sp. mg GAE/gTPC activities of the ELF samples showed similar results to *Usnea* sp. As a result, it was found that the majority of ELF extracts had lower TPC activities than their host lichen species. The reason for this finding may be due to exposure of lichens to various environmental factors such as temperature, pressure, light, and wind in their surrounding environments. These different environmental conditions provide the production of secondary metabolites of lichens and increase their bioactivity. Due to the high phenolic contents of T20-B02 and T20-P26 isolated from *B.capillaris* and T22-B07 ELF samples isolated from *Usnea* sp., we may suggest that they can be used as potential sources in cosmetic industry.

When the total flavonoid content (TFC) was examined, *L. pulmonaria* did not show any TFC activity, but the TFC activities of ELF extracts isolated from this species were found to be in the range of 204.75–399.33 mg rutin/g. Similarly, no results were found for *B. capillaris*, but the amount of flavonoids in the ELF extracts isolated from these lichens ranged from 51.45–373.93 mg rutin/g. Although the TFC value of *Usnea* sp. was found to be 263.32 mg rutin/g, the TFC values of ELF extracts isolated from this species were lower than those isolated from the lichen.

Lichen samples are a natural source of antioxidants.[38,39] However, their slow growth in nature limits their active use in industries such as cosmetics, despite their high antioxidant activity. For this reason, it is thought that it is important to determine the bioactivities, especially the antioxidant activities, of ELF samples isolated from lichens. In particular, it has been reported that the antioxidant activity of the (3R)-5-hydroxymellein (150) compound of ELF000039 species isolated from *Parmotrema austrosinense* lichen was found to be high with an IC50 value of 1,170.8 μg/mL.[40] Also, two new polyketides from *Curvularia trifolii* isolated from *Usnea* sp. exhibited antioxidant activities with IC50 values of 4 and 1.3 mg/ml, respectively.[41] However, studies on the bioactivities of ELF samples are limited, and further detailed researches are needed.

Poornima *et al.* investigated the total antioxidant and phenolic contents of ELF extracts isolated from different lichen species. They reported varying results in the total antioxidant activities (10–90%) of ELF extracts isolated from different lichen species.[42] They also reported similar results in the total phenolic contents to those of the total antioxidant activities. Similarly, the total antioxidant, phenolic, and flavonoid contents of ELF extracts isolated from three different *Usnea* lichens were determined. They reported that the ELF extracts had strong antioxidant activity, while the total phenolic and flavonoid content results showed differences.[43] According to the literature, there is a positive correlation between antioxidant activity and phenolic content.[44,45] Similar to this data, we also observed a positive correlation between TPC of T20-B02 and T20-P26 isolated from the species *B.*

capillaris, and T22-P07 and T22-B07 isolated from *Usnea* sp. and their antioxidant activities. In addition, flavonoid contents prevent the formation of free oxygen radicals caused by UV. In this study, it is anticipated that the high flavonoid levels of ELF extracts isolated from *L. pulmonaria* species between 204.75–399.33 mg rutin/g can be used as supporting agents in cosmetic products for protection against the harmful effects of UV rays. The differences among species may be due to environmental factors such as the host plant, temperature, light, and wind, as well as the concentration of the extracts used in the experiment. Our study suggests that some endolichenic fungi isolated from lichens have higher antioxidant, phenolic, and flavonoid contents than the lichens themselves, making them a potential source of natural antioxidants for the cosmetics industry. Specifically, *B.capillaris* isolated T20-B02, T20-P26, and *Usnea* sp. isolated T22-B07 could be produced in the laboratory and used as sustainable natural sources of antioxidants in the cosmetics industry.

IN VITRO SUN PROTECTION ACTIVITY

"SPF is a measure of how much solar energy (UV radiation) is required to produce sunburn on protected skin (i.e., in the presence of sunscreen) relative to the amount of solar energy required to produce sunburn on unprotected skin. As the SPF value increases, sunburn protection increases." Furthermore, SPF number is important data for quantifying the effectiveness of sunscreen.[46] SPF values are categorized as a minimum (2–12), moderate (12–30), and high ($\geq$ 30).[47] In the cosmetics industry, herbal extracts have been used as sunscreen for a long time.[48] However, lichens have adapted to live in a variety of hazardous conditions, including high UV radiation levels.[49] To protect them from the damaging effects of UV rays, they produce a variety of special metabolites during this process that have antioxidant and photoprotective properties.[50,51] It has been reported that lichen compounds may be good candidates for potent UV blockers due to their wide variety of photoprotective metabolites. For example, pulvinic acid derivatives obtained from lichen specimens protect against UVA, while usnic acid protects against UVB.[52] Some lichen species live in regions with intense UVR. This suggests that lichens, like plants, can be considered an alternative source as they synthesize unique metabolites to protect themselves under intense UV.[53] Lichens can be preferred as an alternative to plants for sunscreen creams due to their protective properties.

In the present study, SPF values of the tested lichen species and ELF extracts from isolating them (1 mg/mL) were determined. Rutin, used as a standard in the experiments, showed an SPF number of 32.11. SPF values of tested lichen species and isolated ELF extracts are shown in Table III. SPF values of lichen samples were 31.45 for *L. Pulmonaria* and 31.78 for *B. capillaris* and *Usnea* sp. It was found to be 31.80 for the patient, showing that the effectiveness of sun protection is high when compared to rutine. On the other hand, these high SPF values detected in lichen extracts could not be detected in the isolated ELF extracts from *L. pulmonaria*, T04-P01 and T04-P13, and remained at low SPF values of 6.54 and 9.44, respectively. For the T04-P03 extract from *L. pulmonaria*, sun protection activity was found be moderate (29.91). The extracts from *B. capillaris*, T20-P07 (7.17) and T20-P10 (8.41), had minimum SPF values, but T20-B02 (29.56), T20-P26 (30.46), and T20-P27 (29.36) had moderate to high SPF values. ELF extracts from *Usnea sp.*, T22-P07 (32.01) and T22-B07 (30.55), were found to have high SPF values.

Overall, the SPF values of the three different lichen extracts evaluated in this study are quite high. Especially, the T04-P03, T20-B02, T20-P26, T20-P27, T22-P07, and T22-B07

Table III
SPF of Lichen and ELF

Name of the lichen and ELF	SPF
Rutin (1 mg/mL standard)	32.11 ± 0.07
Lobaria pulmonaria	31.45 ± 0.04
T04-P01	6.54 ± 0.01
T04-P03	29.91 ± 0.04
T04-P13	9.44 ± 0.01
Bryoria capillaris	31.78 ± 0.03
T20-B02	29.56 ± 0.02
T20-P07	7.17 ± 0.04
T20-P10	8.41 ± 0.02
T20-P26	30.46 ± 0.08
T20-P27	29.36 ± 0.01
Usnea sp.	31.80 ± 0.05
T22-P07	32.01 ± 0.04
T22-B07	30.55 ± 0.02

extracts were found to have *in vitro* SPF values of above 29 at a concentration of 1 mg/mL, indicating that they could have moderate to high potential as a sunscreen ingredient.

Lohezic-Le Dévéhat *et al.*[54] conducted studies on various lichen species, including *C. islandica*, *U. hirta*, and *L. pustulata*, and their natural UV protective activities associated with molecules such as lobaric acid, atranorin, usnic acid, and gyrophoric acid. Hur *et al.* reported the natural UV protective activity of an ELF type compound, (3R)-5-hydroxymellein, isolated from the *P. austrosinense* lichen.[40] In a similar study, Zhao *et al.* isolated a partially purified secondary metabolite, 7-hydroxy-2-octenoic acid ethyl ester (7E), from *Menegazzia terebrata* lichen, coded ELF000548.[55] They reported that the 7E metabolite has high *in vitro* antioxidant content and inhibits melanin synthesis, thereby preventing damage caused by UVB radiation. As a result, they reported that it has potential as a UV protectant for future studies. While the photoprotective activities of certain lichen species are known, studies on the UV protective activities of ELF compounds isolated from lichens are limited. Studies have suggested a positive correlation between SPF and phenolic content, but no similar relationship was observed between antioxidant and flavonoid content.[56]

Similar to the literature, the SPF activities of ELF species with high phenolic content were also found to be high in our results. Especially, the phenolic content of T20-B02 strain isolated from *B.capillaris* was found to be 128.87 and its SPF value was 29.56, while the T20-P26 strain had a similar phenolic content of 129.37 and an SPF value of 30.46. In addition, antioxidant activities of these two species were also observed to be high. However, although the SPF value of T20-P27 was 29.36, its phenolic and antioxidant content were demonstrated to be low. The SPF values of T22-P07 and T22-B07 ELF extracts isolated from *Usnea* sp. were measured as 32.01 and 30.55, respectively, and their phenolic contents were close to the isolated lichen species.

Our study has demonstrated that lichen species and their ELF extracts may possess natural UV protective properties, which can potentially be utilized in the development of cosmetic products. Specifically, the T20-B02 and T20-P26 ELF extracts exhibited a strong positive correlation between their SPF values and total phenol content, suggesting their potential

Table IV
Tyrosinase Inhibition Activity of Lichen and ELF Extracts

Name of the lichen and ELF	Tyrosinase % inhibiton at 1 mg/mL
Kojic acid (1 mg/mL standard)	**45.63 ± 0.003**
Lobaria pulmonaria	Nd
T04-P01	Nd
T04-P03	14.67 ± 0.006
T04-P13	23.37 ± 0.005
Bryoria capillaris	Nd
T20-B02	Nd
T20-P07	33.22 ± 0.001
T20-P10	38.44 ± 0.001
T20-P26	Nd
T20-P27	2.49 ± 0.003
Usnea sp.	Nd
T22-P07	Nd
T22-B07	33.78 ± 0.002

ND: No data

as a natural sunscreen ingredient. These findings are consistent with previous literature on the photoprotective properties of lichen species and their compounds.

TYROSINASE INHIBITION ACTIVITY

Continuous exposure to UV rays is known to cause skin pigmentation. Therefore, tyrosinase inhibitors are used in cosmetic products, especially in skin whitening products.[57] Products naturally containing tyrosinase are often preferred. The tyrosinase inhibitor activity of lichen species has been proven by previous studies.[58,59] However, studies on tyrosinase inhibitor activities of ELF samples isolated from lichens are limited. Determining the tyrosinase inhibitory activities of ELF samples is thought to have the potential to be used in skin whitening products, especially in the cosmetic industry.

In this study, potential tyrosinase inhibitory activities of three different lichen extracts (1 mg/mL) and isolated ELF extract (1 mg/mL) were determined *in vitro* and the results are given in Table IV. Among the tested lichens and their ELF extracts, *L. pulmonaria* and T04-P01, *B. capillaris* and T20-B02, T20-P26, *Usnea* sp., and T22-P07 were found to have no detectable tyrosinase inhibitory activities at the tested concentration. However, T04-P03, T04-P13, T20-P07, T20-P10, and T22-B07 extracts showed varying degrees of tyrosinase inhibition. T04-P03 and T04-P13 exhibited 14.67–23.37% inhibition, respectively, while T20-P07 and T20-P10 showed 33.22–38.44% inhibition, respectively. T22-B07 also demonstrated significant tyrosinase inhibitory activity at 33.78%. The highest tyrosinase inhibitor activity was found to be in the ELF extracts obtained from *B. capillaris*, specifically for the T20-P10 sample.

Higuchi *et al.* reported that the lichens *Hypogymnia physodes*, *Letharia vulpina*, and *Cetraria juniperina* exhibited strong tyrosinase inhibitory activity, and that the mycobiont partner of *H. physodes* showed higher activity than the lichen itself.[60] Verma *et al.* reported that extracts obtained from lichen symbionts of *Arthothelium awasthii* had higher tyrosinase inhibitory activity (67.2%) when compared to *Heterodermia podocarpa* and *Parmotrema tinctorum*.[61] Kim

and Cho reported that the methanol extract of *U. longissima* reduced melanin levels in human melanoma cells through its tyrosinase inhibitory activity.[62] Similarly, Aydın *et al.* investigated the tyrosinase inhibitory activity of methanol and ethyl acetate extracts of *U. longissima* lichen and detected tyrosinase inhibitory activity at increasing concentrations for both solvents.[63] *P. austrosinense* lichen. In other studies, *Phanerochaete sordida* ELF extracts obtained from *Bactrospora myriadea* lichen exhibited moderate tyrosinase inhibitory activity, and a similar study reported that various ELF extracts obtained from 29 different lichens showed varying levels of tyrosinase inhibitory activity, with particularly high activity reported for *Chaetomium globosum* extract (308.4 ± 2.49 μg/mL), *Hypoxylon lividipigmentum* (121.2 ± 2.55 μg/mL), and *Cytospora xylocarpi* (68.50 ± 0.34 μg/mL). The authors reported that C*. globosum* and *H. lividipigmentum* exhibited higher activity compared to the positive control, kojic acid.[64,65]

Based on previous studies, it is known that several species of lichens exhibit tyrosinase inhibitory activity.[58,63] However, in our study, no tyrosinase inhibitory activity was detected in the extracts of three different lichen species tested at a concentration of 1 mg/mL. However, it was found that the isolated ELF species exhibited tyrosinase inhibitory activity. Specifically, high tyrosinase inhibition activity was detected in T20-P07 (33.22) and T20-P10 (38.44) isolated from the *B. capillaris* species, as well as in T22-B07 (33.78) isolated from *Usnea* sp. From this point of view, considering that lichens reproduce slowly in nature and are limited in number, it does not seem appropriate to use lichens in the cosmetic industry for their tyrosinase inhibitory activity. Instead, it can be predicted that ELFs isolated from lichens can be used as a potential source of tyrosinase inhibitors for in the cosmetic industry, as it is both sustainable and economically viable due to its ease and rapidity of production.

CONCLUSION

In recent years, there has been focus on finding new compounds that can be obtained from natural sources with anti-tyrosinase activity and a high SPF number. Especially considering that consumers prefer more natural cosmetic products, the search for potential natural resources is also important for research and development companies and researchers in the sector. Although plants are used as natural resources, lichens have unique molecules and numerous bioactivities that make them especially important for the cosmetic industry. However, given the slow growth and limited availability of lichens in nature, researchers have turned to ELF. Studies conducted in recent years are thought to be a potential resource for the cosmetics industry, as ELF have unique bioactivities. In our study, we compared the tested parameters (total antioxidant activities, total phenolic and flavonoid contents, SPF values, and anti-tyrosinase activity) of selected lichens (*Usnea* sp., *L. pulmonaria,* and *B. capillaris*) and isolated ELF samples from them. As stated in the literature, lichens have many positive biological activities as well as some limitations (poor stability, risk of degradation in active components, and toxicity to normal cells). However, these problems are not insurmountable. It is possible to develop a suitable formulation for improving the stability, dispersal, protection, and effectiveness of lichens and ELF. Furthermore, our results revealed that sunscreen products with anti-tyrosinase activity can be obtained from lichen species and their ELF. Encapsulation of these extracts into biodegradable and biocompatible nanoparticles can increase their bioactivity, reduce toxicity, improve water solubility, and protect against degradation. For these reasons, despite the promising

results, further studies are needed to evaluate the safety and efficacy of lichen extracts or their compounds in cosmetic formulations. Furthermore, the potential of other ELF compounds isolated from various lichens as natural sunscreens or tyrosinase inhibitors could be explored in detail.

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