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Investigation of Biological Activity of *Squamarina cartilaginea* (With.) P. James Species Distributed in Türkiye

Banu Ceren Değirmenci[1,a], Mustafa Kocakaya[2,b], Selen İlgün[3,c], Gökçe Şeker Karatoprak[4,d], Zekiye Kocakaya[5,e,*], Ahmet Ceylan [6,f]

[1] *Yozgat Bozok University, Institute of Science, 66900, Yozgat, Türkiye*
[2] *Yozgat Bozok University, Boğazlıyan Vocational School, Department of Organic Agriculture, 66900, Yozgat, Türkiye.*
[3] *Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 38039, Kayseri, Türkiye*
[4] *Erciyes University, Faculty of Pharmacy, Department of Pharmacognosy, 38039, Kayseri, Türkiye*
[5] *Kayseri University, Safiye Cikrikcioglu Vocational College, Department of Crop and Animal Production, 38280, Kayseri, Türkiye*
[6] *Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, 38039, Kayseri, Türkiye*
**Corresponding author*

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ABSTRACT

In this study, the methanol extract of *Squamarina cartilaginea*, a species distributed in Türkiye, was investigated for its antimicrobial, antioxidant, cytotoxic, and DNA protective effects. The chemical composition of the extract was elucidated through spectroscopic determination of total phenols, total flavonoids, and chromatographic quantification of usnic acid. Antimicrobial activity was assessed using the disk diffusion method, revealing a significant zone of inhibition with a diameter of 17.5 mm against *M. luteus* and *S. aureus*. The antioxidant activity was evaluated through scavenging activities against DPPH and ABTS radicals, demonstrating a concentration-dependent potent scavenging activity against ABTS radicals. Cytotoxic activity was determined using the MTT method on DU-145 (Human Prostate Cancer Cell Line) and Colo 205 (Human Colon Cancer Cell Line) cell lines. The extract exhibited strong cytotoxic activity against the Colo 205 cell line, with a viability percentage of 33.16±2.01 at a concentration of 3.906 µg/mL. Furthermore, the *S. cartilaginea* extract demonstrated DNA protective activity on pBR322 plasmid DNA against UV and H_2O_2 exposure.

Keywords: Cytotoxicity, Lichen, pBR322, *Squamarina,* Usni acid.

[a] *bckkocaman@gmail.com* *https://orcid.org/0009-0000-6777-5415*
[b] *mustafa.kocakaya@yobu.edu.tr* *https://orcid.org/0000-0003-2306-8094*
[c] *erturkselen@gmail.com* *https://orcid.org/0000-0002-8544-0683*
[d] *gskaratoprak@gmail.com* *https://orcid.org/0000-0001-5829-6914*
[e] *zekiyekocakaya@kayseri.edu.tr* *https://orcid.org/0000-0001-5248-0462*
[f] *ahmtcyln7@gmail.com* *https://orcid.org/0000-0002-3087-066x*

Introduction

Lichens are symbiotic associations that generally consist of two partners: a mycobiont (fungus) and a photobiont (algae or cyanobacteria). The organism takes on a thallus-shaped structure and can engage in photosynthesis due to the fungal tissue possessed by algal cells [1]. Lichens, along with phytochemicals, constitute a crucial framework for drug development in modern medicine, especially in developing countries. Presently, numerous scientific studies are underway to identify active compounds derived from plants and other organisms. Lichens, being symbiotic organisms, also produce a variety of secondary metabolites. The chemical diversity of lichen secondary metabolites positions them as potent natural resources for potential applications in medical fields [2]. Additionally, lichens are of interest due to their potent secretions that exhibit therapeutic effects against certain diseases [3].

Lichens have been employed in traditional medicine across diverse civilizations for centuries, owing to their intrinsic biological properties [4]. Utilized both as a source of sustenance and dyes during periods of scarcity, lichens have played a multifaceted role in historical contexts [5]. Throughout history, lichens have found applications across various domains, particularly in medicine, pharmacy, and the chemical industry. In the realm of medicine, their therapeutic properties have been to address ailments such as ringworm, arthritis, constipation, infections, kidney diseases, leprosy, pharyngitis, and rabies [6]. In the context of Turkish folk medicine, albeit less common than the recognized healing properties of plants, lichens are reported to be utilized in treatments [7].

In addition to the traditional applications of lichens in folk medicine, the discovery that certain lichen compounds contribute to UV-B protection, the bioplastic degradation capacity in frozen foods, and the presence of antifreeze proteins with potential implications for preventing desertification are noteworthy findings that further enhance the distinctive biological profile of lichens [8]. Numerous secondary metabolites produced by lichens exhibit potential antioxidant properties owing to their aromatic structures [9,10]. It is well-established that algae, bacteria, fungi, plants, and lichen species synthesize compounds with antimicrobial effects as a defense mechanism in nature [11].

The genus *Squamarina*, inclusive of the species *Squamarina cartilaginea*, manifests itself in calcareous soil and rocks through the formation of spreading lobes or overlapping scales, lacking a well-developed upper and lower cortex [12]. Widely distributed, this genus

encompasses 28 species [13]. Although chemical studies on the *Squamarina* genus are limited, one identified compound is the naphthoquinone squamaron [14]. Several investigations into the *Squamarina* genus reveal the inhibitory effects of usnic acid extract from *Squamarina lentigera* against bacteria such as *Bacillus megaterium* and *Bacillus subtilis* [15]. Notably, psoromic acid derived from *Squamarina cartilaginea* exhibits robust antibacterial activity against oral pathogens, including *Streptococcus gordonii* and *Porphyromonas gingivalis* [16].

To further explore the therapeutic attributes of lichens, considering the information presented above, this study conducted a comprehensive examination of the antioxidant, antimicrobial, cytotoxic activities, and DNA protective effects associated with the extract derived from *Squamarina cartilaginea*. The chemical composition of the extract, as well as the presence and quantification of usnic acid, were determined through High-Performance Liquid Chromatography (HPLC). This research contributes a thorough investigation into the biological activity potential of *Squamarina cartilaginea*, providing initial data for this species, many of which lack precedent in the existing literature regarding biological activities.

Material and Method

Lichen Species Used in the Study

The species used in this study was collected in Üzümdere Village of Antalya Ibradı, along the road in a mixed woodland of *Quercus* sp. and *Pinus brutia*, located in an area characterized by predominantly limestone bedrock, with coordinates 37° 05' 07.6" N, 31° 39'43.0" E and an altitude of 550 m. This lichen was collected from limestone bedrock in the area because it is commonly found on calcium carbonate-containing substrates, especially limestone and other basic rocks [12]. Floral references such as 'Flechten Flora' [17] and 'The Lichen Flora of Great Britain and Ireland' [18] were consulted in the taxonomic identification of the collected lichen sample. External morphological characters were examined using a stereomicroscope (Olympus SZX16), while anatomical features were investigated with a light microscope (Olympus BX53 Light microscope). Additionally, macroscopic and microscopic digital photographs were meticulously taken using the Olympus DP25 digital camera connected to the Stereomicroscope Olympus SZX16 and Olympus BX53.

Critical parameters such as spore sizes, ascus sizes, hymenium heights, and paraphysis widths played a decisive role in the meticulous categorization of species and subspecies. These morphological features were precisely measured utilizing a micrometer, and chemical reagents were judiciously applied for diagnostic purposes. The specimens were systematically recorded and systematically stored in envelopes, thus constituting herbarium materials housed at Yozgat Bozok University, Boğazlıyan Vocational School, Lichen Herbarium [Leg. and Det.: Mustafa Kocakaya, herbarium number: MK0365].

Preparation of Extracts

Fifteen grams of lichen sample were subjected to extraction using 80% methanol in a water bath at 37 ºC for 8 hours, with intermittent shaking during three cycles. Methanol solution is a widely used and effective solvent for the extraction of natural antioxidants, especially phenolic compounds, from plant materials. Therefore, the extraction was performed with methanol [19]. The filtrates obtained after each methanol treatment were consolidated and concentrated under vacuum using a rotavapor (maintained at 37-38 ºC). Subsequently, all obtained extracts underwent lyophilization and were dried, then preserved at -20 ºC until analysis [20].

Chemical Composition

Determination of total phenol amount

The total phenolic content of the extract was determined as gallic acid equivalent using the Folin-Ciocalteu method [21]. For this, a sample solution (100 µL) was mixed with Folin-Ciocalteu reagent (500 µL) in the presence of 6 mL of distilled water within a 10 mL container. After one minute, 1.5 mL of 20% aqueous Na_2CO_3 was added, and the volume was adjusted to 10 mL. The control group used a reagent mixture without the extract. Following a 2-hour incubation at 25 ºC, absorbance values at a wavelength of 760 nm were measured, and these values were compared with the gallic acid calibration curve. Each experiment was replicated three times, and the results are presented as mean values.

Determination of total flavonoid amount

The total flavonoid content in the extract was determined following the method proposed by [22]. In this procedure, 1 mL of extract was mixed with 0.3 mL of 5% $NaNO_2$ solution at the initial moment (t=0). After five minutes (t=5) 0.3 mL of 10% $AlCl_3.6H_2O$ solution was added, and after six minutes (t=6) 2 mL of 1 M NaOH solution was added. The mixture was then prepared by adding 2.4 mL of water. Absorbance measurements at 510 nm wavelength were conducted, and the total flavonoid content in the extract was quantified as mg $_{CA}$ /g $_{ekstre}$, equivalent to catechin (CA). The catechin calibration curve was established using ethanol. All measurements were conducted in triplicate, and the results were reported as average values.

Quantification of usnic acid by high pressure liquid chromatography (HPLC)

The lichen extract was dissolved in 80% methanol and analyzed using Shimadzu LC-20AT HPLC system. PDA spectrophotometric detector was employed in the analyses, and the solvent of choice was methanol-water-phosphoric acid (75:25:0.9, v/v/v). The flow rate was set at 1 mL/min, and the usnic acid standard was dissolved in 0.5% DMSO (Dimethylsulfoxide).

Determination of Antimicrobial Activity

In this study, the antimicrobial activity of the *Squamarina cartilaginea* extract was investigated against Gram-negative bacteria, including *Proteus mirabilis* (ATCC 25933), *Escherichia coli* (ATCC 25922), *Enterobacter aerogenes* (ATCC 13048), and Gram-positive bacteria, such as *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 10240), and *Staphylococcus aureus* (ATCC 25923), using the disk diffusion method. The *S. cartilaginea* extract, prepared at a concentration of 20 mg/mL in DMSO, was filtered through sterile filters with a diameter of 0.45 µm. The resulting extract was impregnated onto sterile discs (Oxoid, blank disc) in a volume of 20 µL.

Bacterial strains were cultured by incubating at 37 °C for 24 hours in Mueller-Hinton Broth (MHB) medium, and their concentrations were adjusted to $1.5x10^8$ cfu/mL using a UV-VIS Spectrophotometer according to the 0.5 McFarland standard. Bacterial cultures were spread on Mueller-Hinton Agar (MHA) medium in volumes of 100 µL. Discs containing the lichen extract and control groups were placed onto the medium with bacteria and then incubated at 37 °C for 24 hours. Ampicillin (amp) standard antibiotic discs (10 µg/disc) were used as a positive control, and discs impregnated with sterile DMSO served as the negative control. The diameters of the inhibition zones resulting from incubation were measured using a millimetric ruler. The experiment was conducted in triplicate, and the arithmetic averages were calculated to assess the obtained results.

Determination of DNA protective activity

To assess the efficacy of the *S. cartilaginea* extract in safeguarding DNA from UV and oxidative-induced damage, pBR322 plasmid DNA (Thermo Fisher Scientific) was employed. Plasmid DNA was subjected to damage induced by H_2O_2 and UV exposure in the presence of the lichen extract. Sample preparations consisted of 5 µL of lichen extract at a concentration of 20 mg/mL, diluted by 5%, plasmid 3 µL, dH_2O 6 µL, and H_2O_2 1 µL. The samples were organized as follows:

I. In Tube: pBR322
II. In Tube: pBR322 + UV
III. In Tube: pBR322 + H_2O_2
IV. In Tube: pBR322 + UV + H_2O_2
V. In Tube: pBR322 + *S. cartilaginea* extract + UV + H_2O_2.

After exposing the tubes containing the lichen extract to UV light for 5 minutes, incubation was carried out for 3 hours. Subsequently, 3 µL of loading dye was added, and the mixture was run on a 1% agarose gel at 80 V for 3 hours. The gel was then photographed using a gel imaging system (Bio-Rad ChemiDoc™ XRS+), as described by [23].

Evaluation of cytotoxic effects of extracts by MTT (3-(4,5-dimethylthiazol–2-yl)-diphenyl tetrazolium bromide) method

In this study, DU-145 (Human Prostate Cancer Cell Line) and Colo 205 (Human Colon Cancer Cell Line) cell lines were employed to assess the cytotoxic effects of the extract on cancerous cell lines. Throughout the experiment, the cells were maintained in cell culture dishes with ventilated lids in a CO_2 incubator at +37 °C. Regular observations were conducted, the medium was refreshed every two days, and the culture was sustained.

The cytotoxic effects on DU-145 and Colo 285 cell lines were determined using the MTT colorimetric method with an 80% methanol extract. Twenty-four hours prior to the experiment, the cells in the flask were counted, and 10000 cells in 100 µL were seeded into each well. After 24 hours of incubation, the supernatant above the adhered cells was removed, and the extract, prepared by diluting in the medium, was added to the plate in volumes ranging from 3.906 to 1000 µg/mL. Subsequently, the plate was placed in a carbon dioxide incubator at 37 °C for 48 hours. An MTT working solution was prepared at a concentration of 0.5 mg/mL from the stock MTT solution, dissolved in sterile PBS, and added to the 96-well microplates. After the incubation period, the medium in the plate was aspirated, and 100 µL DMSO was added. Following shaking for 5 minutes, the optical densities of the cells in the plates were measured at a wavelength of 540 nm using an ELISA device (Bio-Rad, USA). The average absorbance values from the control wells were taken, considering this value as 100% live cells. The absorbance values obtained from the wells treated with the solvent and extract were calculated as percent viability by proportioning to the control absorbance value.

The percentage of cell viability was calculated using the formula: (Concentration O.D. / Control O.D.) × 100.

Determination of Antioxidant Activity

1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging capacity evaluation

A 50 nM pH 7.4 Tris-HCl buffer was mixed with 1 mL of 1,1-diphenyl-2-picrylhydrazyl solution prepared in 0.1 mM methanol. A reagent mixture without the extract was used as a control, and butyl hydroxytoluene (BHT) was included as a positive control. Following a 30-minute incubation at room temperature and in darkness, absorbances were measured at a wavelength of 517 nm.

The percentage of inhibition was calculated using the following formula. Analyses were conducted in four independent replicates, and the results were averaged [24].

% inhibition = [(Abs Control - Abs sample) / Abs control] x 100

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging capacity evaluation

The $ABTS^{\bullet+}$ radical, with a concentration of 7 mM, was generated by incubating it in dark conditions for 12-16 hours in its aqueous solution and $K_2S_2O_8$ (2.45 mM, final concentration). The absorbance of the resulting solution was adjusted to 0.700 (± 0.030) at 734 nm at room temperature. Subsequently, this radical solution (990 µL) and extract solutions (10 µL) were meticulously mixed,

and the reaction kinetics were measured at 734 nm wavelength for 30 minutes with 1-minute intervals. Concentration-dependent inhibition percentages were calculated as Trolox equivalent (TEAC) using the acquired data. The experiments were conducted three times in parallel, and the results were averaged [21].

Results and Discussion

Squamarina cartilaginea, a widely distributed species belonging to the genus *Squamarina*, has been the subject of limited studies investigating its biological activities. In this study, the total phenol, total flavonoid content, and usnic acid—a common lichen acid—in the methanol extract obtained from *Squamarina cartilaginea* were determined using high-pressure liquid chromatography. Furthermore, the study elucidated the antimicrobial, DNA protective, cytotoxic, and antioxidant activities of the extract.

The total phenol and total flavonoid content of the extract were determined using spectrophotometric methods. The total phenol amounts are expressed in gallic acid equivalents, and the total flavonoid amounts are expressed in catechin equivalents. The total phenol content of the lichen extract was measured as 44.72±4.67 mg_{GAE}/g_{exract}, while the total flavonoid content was determined as 30.10±3.28 mg_{CA}/g_{exract} (Table 1). When examining studies that investigated the phenolic substance content in various lichen samples, variations were observed among different species [25,26].

Table 1. Total phenol, total flavonoid and usnic acid amounts in *S. cartilaginea* extract

Extract	Total phenol [mg_{GAE}/g_{exract}]	Total flavonoid [mg_{CA}/g_{exract}]	Usnic acid (mg/g_{exract})
S. cartilaginea	44.72±4.67*	30.10±3.28*	451.637±0.00

*Data are expressed as mean ± standard error (n=3)

Since its first isolation in 1844, usnic acid has been a frequently studied secondary metabolite in lichens. A variety of interesting biological and physiological activities have been determined in pharmacology and clinical studies [27,28].The usnic acid content of the *Squamarina cartilaginea* extract was calculated as 451,637 mg/g_{exract} (Table 1). In the literature, the amount of usnic acid in the acetone extract of *Squamarina lentigera*, another species of the genus, was determined to be 2.47±0.01% [15]. The amount of usnic acid in the extract prepared with a mixture of dichloromethane and methanol (1:1) was determined as 345.098 m/z [29]. The calibration equation and correlation coefficient in the method using usnic acid as a standard are provided in Table 2. The chromatogram of the lichen extract is presented in Figure 1.

Table 2. Calibration equation and correlation coefficient of the usnic acid standard

Material	Calibration Equation [y=ax+b]	Correlation Coefficient [r^2]
Usnic acid	y=23.68971x+94.49502	0.99970

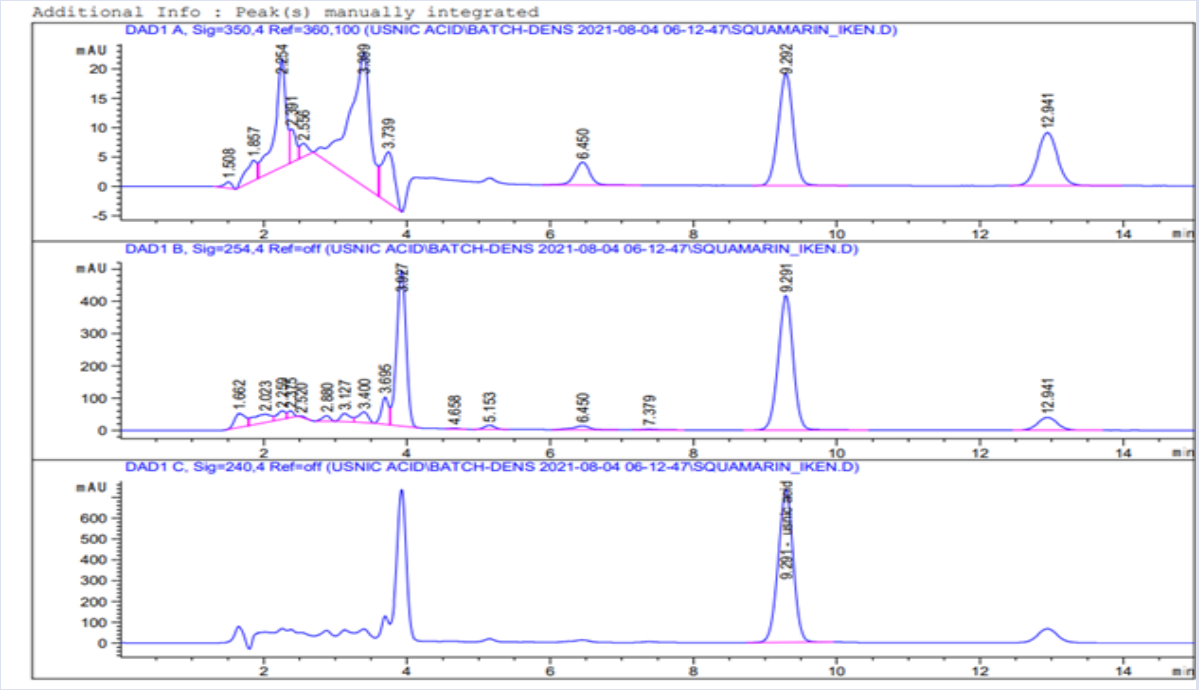

Figure 1. Chromatogram of *S. cartilaginea* extract

The antimicrobial effects of the *S. cartilaginea* extract against six bacterial strains were tested, and the resulting inhibition zones are presented in Table 3. Ampicillin, used as a positive control, exhibited higher activity than the lichen extracts, while no activity was observed in DMSO, used as a negative control.

Table 3. Antimicrobial activity of *S. cartilaginea* extract

Microorganism	*Squamarina cartilaginea*	(-) Control empty disk	(+) Control (Ampicillin)
E. coli	10.0 mm	-	16.0 mm
P. mirabilis	9.0 mm	-	24.0 mm
M. luteus	17.5 mm	-	30.0 mm
E. aerogenes	9.0 mm	-	12.0 mm
S. aureus	17.5 mm	-	20.0 mm
B. subtilis	9.5 mm	-	14.0 mm

Photographs of the inhibition zones created by the extract are presented in Figure 2. The *S. cartilaginea* extract exhibited the highest antimicrobial activity with a value of 17.5 mm against *M. luteus* and *S. aureus* strains, and the lowest activity with a value of 9.0 mm against *P. mirabilis* and *E. aerogenes* strains. In a study, it was determined that the *S. cartilaginea* methanol extract was active against *Enterobacter cloacae* up to 2000 µg/mL and affected fungal hyphae morphologically and lysosome activity against *Staphylococcus aureus*. Thus, this species has been shown to be a potentially bioactive source of compounds with antibacterial properties. In various studies, findings have indicated a correlation between the amount of usnic acid and antimicrobial activity, emphasizing that antimicrobial activity increases with the concentration of usnic acid. This highlights the importance of investigating lichens in detail as species of medical significance [15,30].

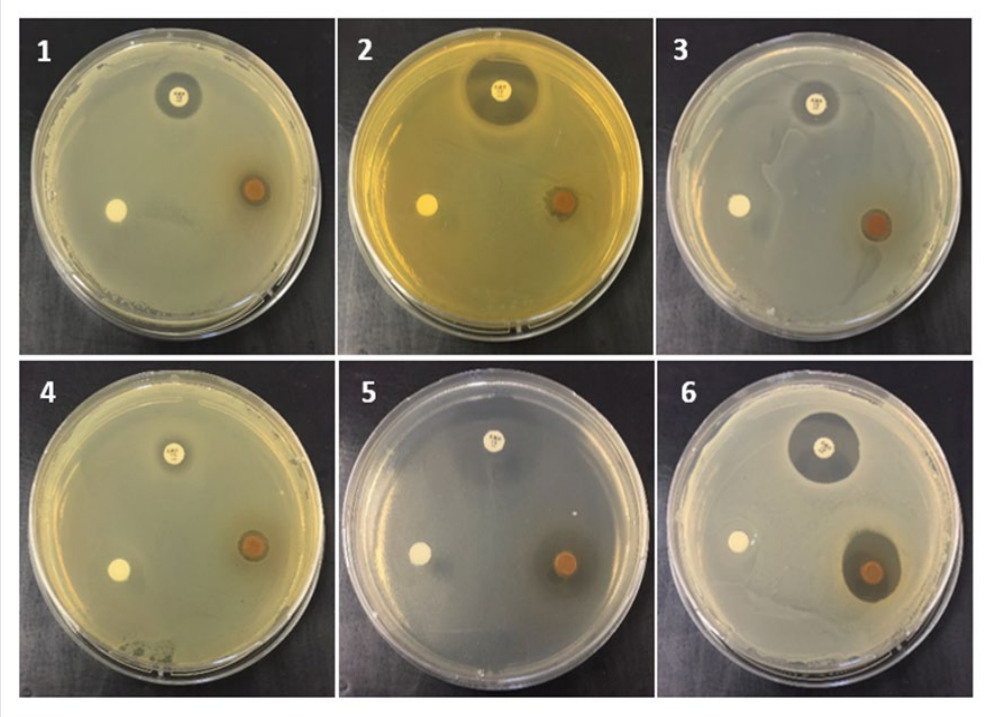

Figure 2. Antimicrobial activities of S. cartilaginea extract 1. E. coli, 2. P. mirabilis, 3. B. subtilis, 4. E. aerogenes, 5. M. luteus, 6. S. aureus

The DNA protective activity of the *S. cartilaginea* extract was assessed using pBR322 plasmid DNA, which exists in Form I (supercoil structure) and Form II (open ring structure). Form III refers to plasmid DNA in a linear structure resulting from the double-strand break in plasmid DNA. Standard pBR322 was loaded in well 1 and used as a marker. When the PBR322 plasmid DNA was exposed to UV and H_2O_2, damage occurred, alterations in Form I and Form II structures were observed, and the emergence of Form III structure was noted, as seen in the 4th well. When tested against UV or H_2O_2 individually, a decrease in the intensity of bands in Form I structure and an increase in the intensity of bands in Form II structure were observed.

In the 5th well, where the *S. cartilaginea* extract was added to the reaction mixture, it was observed that linear DNA formation was suppressed, and the structure of supercoiled DNA was preserved. Based on the obtained results, the lichen extract exhibited DNA protective activity against UV and H_2O_2 (Figure 3). The evaluation of the DNA protective effect of the *S. cartilaginea* species extract was conducted for the first time in this study, and no relevant data were found in the literature.

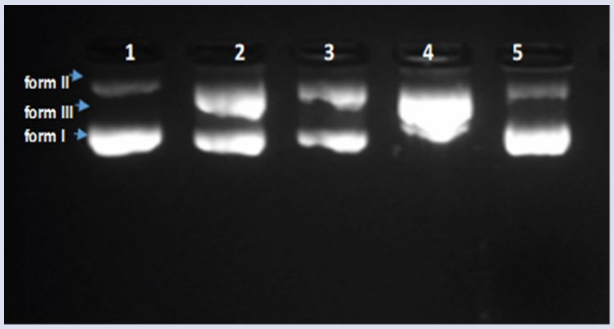

Figure 3. PBR322 DNA Cleavage agarose gel electrophoresis image. 1. pBR322, 2. pBR322 + UV, 3. pBR322 + H_2O_2, 4. pBR322 + UV + H_2O_2, 5. pBR322 + *S. cartilaginea* extract + UV + H_2O_2

As a result of the studies conducted to determine the cytotoxic effects of the *S. cartilaginea* species on DU-145 and Colo 205 cell lines, it was observed that the extract exhibited a high cytotoxic effect on both cell lines. The cytotoxic effect on DU-145 and Colo 205 cell lines for this lichen species was elucidated for the first time in this research. In the DU-145 cancer cell line, the extract reduced the viability of the cancer cell line to less than 50% in the concentration range of 31.25-1000 µg/mL compared to the control (Figure 4) and was observed to be quite toxic. The highest activity was observed at a concentration of 1000 µg/mL with a viability rate of 12.837±0.71%. The cytotoxic effects of the extract were also evaluated on the Colo-205 cell line, and it was found to be more toxic in this cell line compared to Du-145. It was determined that the extract showed high toxicity even at the lowest concentration. While 16.97±0.48% viability was observed at a concentration of 1000 µg/mL, 33.16±2.01% viability was measured at a concentration of 3.906 µg/mL (Figure 5).

When evaluating the cytotoxic effect on cancerous cell lines within the scope of this study, it can be hypothesized that the high cytotoxic activity is related to the usnic acid content in the lichen. However, it is also possible that other compounds are responsible for this effect or that multiple compounds act synergistically to produce this effect. Studies have shown that especially usnic acid, a dibenzofuran derivative, exhibits important pharmacological activities [31]. Regarding antiproliferative activity, it has been reported that both (-) and (+) isomers of usnic acid demonstrate moderate to strong cytotoxicity in vitro against a wide range of murine and human cancer cell lines [31,32,33,34,35,36,37].

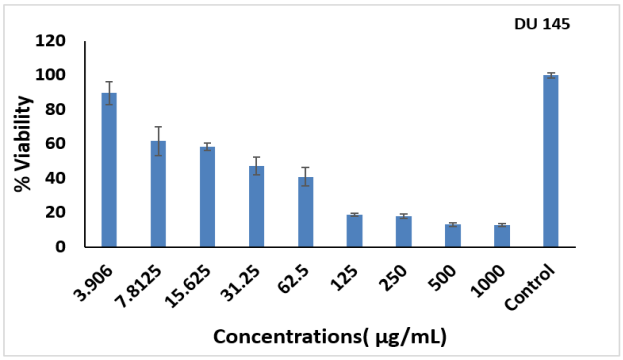

Figure 4. Cytotoxic effects of *S. cartilaginea* extract on DU-145 cells

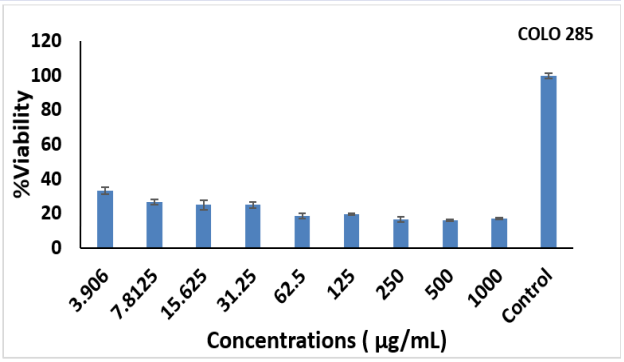

Figure 5. Cytotoxic effects of *S. cartilaginea* extract on Colo 205 cells

It has been observed that the *S. cartilaginea* extract effectively scavenges the DPPH radical in a concentration-dependent manner at physiological pH values. % Inhibition values are provided in Table 5. The extract was not as effective as BHT at the investigated concentrations. It was determined that the *S. cartilaginea* extract has moderate antioxidant capacity. In a study investigating the antioxidant capacity of *S. cartilaginea* and different lichen extracts, it was reported that the methanol extract of *S. cartilaginea* had a higher DPPH radical scavenging potential than other lichen species, and its IC_{50} value was 0.9 µg/mL [38]. When the ABTS radical scavenging effect of the *S. cartilaginea* extract was evaluated, high activity was determined, especially at 4 and 2 mg/mL concentrations (Table 4). However, it was not as effective as standard BHT at low concentrations (0.5 mg/mL and 1 mg/mL).

Table 4. DPPH• and $ABTS^{•+}$ radical scavenging effects

	DPPH• % inhibition			
	0,5 mg/mL	**1 mg/mL**	**2 mg/mL**	**4mg/mL**
S. cartilaginea	8.42±0.68	12.46±0.55	22.27±1.01	44.99±0.98
BHT	73.69±0.2	79.17±0.1	81.0±0.3	86.9±0.1
	TEAC (mmol/L/Trolox)			
S. cartilaginea	0.79±0.16	1.18±0.25	2.10±0.28	2.54±0.25
BHT	2.50±0.1	2.51±0.8	2.54±0.9	2.55±0.9

Conclusion

In conclusion, this study investigated the biological activities of *Squamarina cartilaginea*, a lichen species distributed in Türkiye. Despite its wide distribution, there has been limited research on the biological activities of *S. cartilaginea*. The methanol extract of *S. cartilaginea* exhibited antimicrobial, antioxidant, cytotoxic, and DNA protective activities. In the chemical composition of the extract, the amount of usnic acid was analyzed and the amounts of total phenols and total flavonoids were determined. Compared to a study on *Squamarina lentigera,* a high concentration of usnic acid was found. The amount of usnic acid determined shows the potential to investigate the effects of this compound on biological activities.

Antimicrobial assays demonstrated notable inhibition zones against both Gram-negative (*Escherichia coli*, *Proteus mirabilis*, *Enterobacter aerogenes*) and Gram-positive (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*) bacteria. The extract's efficacy in protecting plasmid DNA from UV and H_2O_2-induced damage further highlights its potential applications.

Cytotoxicity assessments on DU-145 (Human Prostate Cancer Cell Line) and Colo 205 (Human Colon Cancer Cell Line) revealed strong cytotoxic effects, particularly on the Colo 205 cell line. The extract demonstrated concentration-dependent scavenging activity against DPPH and ABTS radicals, indicating its moderate antioxidant capacity.

These findings contribute to the understanding of the biological potential of *Squamarina cartilaginea* and underscore its significance as a source of bioactive compounds. Lichens have garnered considerable attention due to their distinctive secondary metabolites. Consequently, the isolation and large-scale cultivation of lichen mycobionts hold the potential for extracting bioactive compounds, paving the way for the commercial utilization of lichens in the pharmaceutical sciences. This approach not only facilitates the exploration of unique and valuable compounds but also contributes to unlocking the commercial value inherent in lichen-derived products. The endeavor to harness the pharmaceutical potential of lichen secondary metabolites through isolation and mass culture represents a promising avenue for future research and industrial applications.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgment

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Author's Contributions

MK, ZK and BCD designed the current research experiments. BCD, MK, Sİ, ZK, GŞK, and AC performed the experiments. ZK and GŞK wrote and edited the manuscript.

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