

## Antioxidant and Antibacterial Potencies of *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale and *Dermatocarpon miniatum* (L.) W. Mann. Lichens from Black Sea Region in Turkey

*Türkiye’de Karadeniz Bölgesi’ndeki Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale and *Dermatocarpon miniatum* (L.) W. Mann. Likenlerinin Antioksidan ve Antibakteriyel Potansiyelleri

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### Abstract

Antibacterial properties of *Dermatocarpon miniatum* (L.) W. Mann and *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale lichens were investigated by disc diffusion and Minimum Inhibitory Concentration (MIC) methods. Antioxidant capacity of the lichens were examined by utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-Azino-bis (3-ethylbenzenotiazoline-6-sulphonic acid (ABTS) radical scavenging activity, metal chelating activity, total antioxidant capacity, determination of total phenolic and total flavonoid contents. Extracts showed antibacterial effect against all bacteria except for *Escherichia coli* and *Salmonella enterica*. When compared antibacterial efficiency of the tested lichens, it is concluded that *X. conspersa* lichen is more active than *D. miniatum* lichen. DPPH radical scavenging activity of the extracts are increased in the following order: ethanol extract of *X. conspersa* > acetone extract of *D. miniatum* > ethanol extract of *D. miniatum* > acetone extract of *X. conspersa*. Studied lichen extracts showed relatively weak metal chelating activity. According to the obtained results, it is concluded that *D. miniatum* and *X. conspersa* lichen extracts can be alternative antibacterial and antioxidant agents.

**Keywords:** Antibacterial Activity, Antibiotic, Antioxidant Activity, Lichen

### Öz

*Dermatocarpon miniatum* (L.) W. Mann ve *Xanthoparmelia conspersa* (Ehrh. Ex. Ach.) Hale likenlerinin antibakteriyel özellikleri disk difüzyon ve Minimum İnhibitör Konsantrasyon (MİK) yöntemleri ile araştırıldı. Likenlerin antioksidan kapasitesi, 2,2-difenil-1-pikrilhidrazil (DPPH) radikal temizleme aktivitesi, 2,2'-Azino-bis (3-etilbenzenotiazolin-6-sülfonik asit (ABTS) radikali temizleme aktivitesi ve metal şelatlama aktivitesi kullanılarak incelenmiştir. Toplam antioksidan kapasite toplam fenolik ve toplam flavonoid içeriğinin belirlenmesi yöntemleri kullanılarak araştırıldı. Ekstraktlar *Escherichia coli* ve *Salmonella enterica* dışındaki diğer tüm bakterilere karşı antibakteriyel etki göstermiştir. Test edilen likenlerin antibakteriyel etkinliği karşılaştırıldığında, *X. conspersa* likeninin *D. miniatum* likeninden daha aktif olduğu belirlenmiştir. *D. miniatum* liken ekstraktların DPPH radikal temizleme aktiviteleri *X. conspersa*'nın etanol ekstraktı > *D. miniatum*'un aseton ekstraktı > *D. miniatum*'un etanol ekstraktı > *X. conspersa*'nın aseton ekstraktı şeklinde sıralanmıştır. Çalışılan liken ekstraktları oldukça zayıf metal şelatlama aktivitesi göstermiştir. Çalışmadan elde edilen sonuçlar *D. miniatum* ve *X. conspersa* liken ekstraktlarının antibakteriyel ve antioksidan ajanlara alternatif olarak kullanılabileceklerini göstermektedir.

**Anahtar kelimeler:** Antibiyotik, Antibakteriyel Aktivite, Antioksidan Aktivite, Liken

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## 1. Introduction

The outbreak of resistance to pathogenic bacteria to the current antibacterial agents is a very general and threatening trouble around the world. Besides intrinsic ability of bacteria, extrinsic factors cause acquiring resistance of bacteria to antibiotics. These extrinsic factors are using improper and extensive antibiotics and deficiency of appropriate or late diagnosis of infection. Consequently, there is a perpetual demand for brand and effective antibacterial drugs (Rani et al., 2017).

Medicinal plants have been utilized in the treatment of many infectious diseases. Medicinal plants are natural alternatives to antimicrobial agents (Mahesh and Satish, 2008). Antibiotics are occasionally have side effects but using medicinal plants have some benefits like little side effects, relatively cheap and better patient tolerance (Joshi and Sahu, 2014).

Nowadays, there has been a rising demand in utilizing of traditional plants for curative antioxidant agents. An antioxidant can be described as any substance that retards, hinders or eliminates oxidative detriment to a target molecule. Natural antioxidants are known to demonstrate a great deal of biological impacts such as antibacterial, anti-inflammatory, antiviral, antiallergic and anticancer efficiency (Aadesariya et al., 2017).

Recently, most of the antioxidants are produced synthetically. Butylated hydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT) and gallic acid which are synthetic antioxidants are known to possess possible side influence carcinogenicity. Because of this situation, using synthetic antioxidants are restricted. Antioxidant substances derived from plants are reliable and complete the effect of free radicals thus preserving the organism from many different kind of diseases. For this reason, an interest to investigate medicinal plants for the existence of natural antioxidants has risen (Reddey and Grace, 2016).

Lichens are the symbiotic associations including a fungal partner and an algal partner and they are recognize to possess medicinal effects on many illnesses in folk medicine in worldwide (Sharma and Kalikotay, 2012).

Antibacterial and antioxidant properties of lichen species have been recognized for long years. These activities of lichens has been recorded by

several researchers (Rankovic et al., 2010; Buçukoğlu et al., 2013; Sisodia et al., 2013).

Antibacterial and antioxidant activities of acetone and methanol extracts of *Usnea rubratincta*, *Ramalina dumeticola* and *Cladonia verticillata* were investigated and it was found that some extracts inhibited *Staphylococcus aureus* and *Bacillus subtilis*. Moreover, it was found that DPPH activity of the extracts ranged from 16.4 % and 33.09 % (Gunasekaran et al., 2016).

Paudel et al. (2012) searched antioxidant and antibacterial activities of twenty four lichen species. It was found that extracts of twenty one lichen species were active against *Bacillus subtilis* and seven species were active against *Staphylococcus aureus*. Besides, in DPPH assay, *Peltigera* sp., *Cladonia* sp., and *Canoparmelia* sp. exhibited comparable activity with standard antioxidant BHA. However in ABTS assay, extracts of *Parmotermia* sp., *Ramalina* sp., *Peltigera* sp. and *Cladonia* sp. showed stronger activity than standard antioxidant ascorbic acid.

Antioxidant and antimicrobial activities of chloroform, methanol and water extracts of *Cladonia rangiformis* Hoffm. were also screened. Extracts exhibited weak antibacterial activity but no antifungal activity against test microorganisms (Yücel et al., 2007).

*Dermatocarpon miniatum* (L.) W. Mann traditionally used in China to reduce high blood pressure, diuretic, inadequate nutrition in children, dysentery, regulation of digestion and elimination of abdominal distension (Crawford, 2015).

The other lichen species that makes up our work is the *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale. It is utilized in the treatment of snake bites, cuts, treatment of inflammatory gingivitis, and treatment of sore throat (Crawford, 2015).

In this study, we targeted to search antibacterial and antioxidant activities of *X. conspersa* and *D. miniatum* lichens.

## 2. Material and Methods

### 2.1. Lichen Materials

*D. miniatum* and *X. conspersa* specimens used in the study were collected from the following localities which located in Giresun in 2016 (Table 1).

These specimens were dried at room temperature and identified using Smith et al. (2009). A voucher specimens of the lichens (*D. miniatum*: Herb. no: 6396, *X. conspersa* Herb. no: 6397) were kept in the Biology Department, Faculty of Science and Arts, Giresun University, Giresun, Turkey.

**Table 1.** Localities where lichens are collected

<i>D. miniatum</i>	Giresun, E of Giresun Castle, 110 m, 40°55'13" N, 38°23'25 E", 12.03.2006.
<i>X. conspersa</i>	Giresun, Keşap, Değirmenağzı village, sea shore, 2 m, 40°58'23" N, 38°37'29 E", 19.06.2015.

**Table 2.** Bacteria which used in the study.

Bacteria	Gram (+) Bacteria / Gram (-) Bacteria	Where obtained from
<i>Salmonella enterica</i> ATCC 14028	Gram (-)	Giresun Province Control Laboratory
<i>Proteus vulgaris</i> FMC 1	Gram (-)	Firat University
<i>Enterobacter aerogenes</i> CCM 2531	Gram (-)	Firat University
<i>Yersinia pseudotuberculosis</i> ATCC 911	Gram (-)	Rize University
<i>Escherichia coli</i> ATCC 35218	Gram (-)	Giresun University
<i>Klebsiella pneumoniae</i> (laboratory isolate)	Gram (-)	Yeditepe University
<i>Gordonia rubripertincta</i> (lab isolate)	Gram (+)	Yeditepe University
<i>Bacillus megaterium</i> (laboratory isolate)	Gram (+)	Yeditepe University
<i>Staphylococcus aureus</i> subsp. aureus ATCC 25923	Gram (+)	Giresun Province Control Laboratory
<i>Bacillus cereus</i> 702 ROMA	Gram (+)	Firat University
<i>Bacillus cereus</i> 702 ROMA	Gram (+)	Rize University

#### 2.4. Antibacterial Activity of the Lichens

On Mueller-Hinton agar each bacterial inoculum was swab streaked that has been formerly arranged by inoculating bacterial strains into nutrient broth with overnight incubation. Crude extracts were dissolved with DMSO at 15 mg/mL. Then, they were sterilized by using 0.45 µm pore sized filter. The discs were put into agar plates and filled with 25 µL ethanol extract of *D. miniatum*, 25 µL acetone extract of *D. miniatum*, 25 µL ethanol extract of *X. conspersa*, 25 µL acetone extract of *X. conspersa* and 25 µL DMSO. Tetracycline and gentamycin discs were utilized as standard antibacterial agents. Plates were then incubated for 24 h at 37 °C. The clear zone of inhibition was observed and measured in mm (Murray et al., 1995; Šarić et al., 2009).

#### 2.5. Determination of MIC

The MIC was defined by the broth tube dilution method (NCCLS, 1993). A series of dilutions with concentrations ranging from 15 to 0.02929 mg/mL for extracts was utilized in the assay against each microorganism tested. Extracts

#### 2.2. Test Microorganisms

Six gram negative and five gram positive bacteria were utilized in the current study.

#### 2.3. Extract Preparation

15 g of the powdered sample were extracted with Soxhlet apparatus utilizing 150 mL acetone and ethanol solvents, separately. The extraction process followed by filtration with Whatman filter paper no 1. The filtered extract concentrated in vacuo at 40°C using a rotary evaporator. Extracts were kept at -80°C for other tests (Kumar et al., 2012).

dissolved in DMSO. Two-fold dilutions of extracts were prepared in Mueller-Hinton broth in test tubes (Marijana et al., 2010).

#### 2.6. Antioxidant Capacity of *X. conspersa* and *D. miniatum*

##### 2.6.1. Total Phenolic Content

Total phenolic content of lichen extracts was determined by the procedure of Slinkard and Singleton (1977) using gallic acid standard. The absorbance was measured at 760 nm. The quantity of the total phenolic compounds was denoted as µg gallic acid equivalent (GAE)/mL. The tests were carried out three times. The tests were carried out three times.

##### 2.6.2. Total Flavonoid Content

Total flavonoid content of lichen extracts was determined by the method of Zhishen et al. (1999). Absorbance was read spectrometrically at 510 nm. The amount of total flavonoid compounds was calculated as µg catechin

equivalent (CE)/mL. The tests were carried out three times.

### 2.6.3. Metal Chelating Activity

Lichen extracts and standard (EDTA) were prepared at concentrations of 250-1000 µg/mL. The absorbance was measured at 562 nm (Loizzo et al., 2012). Results are calculated from the following equation:

$$\% \text{ Activity: } \left[ \frac{A_0 - A_1}{A_0} \right] \times 100 \quad (1)$$

A<sub>0</sub>=Absorbance of control

A<sub>1</sub>= Absorbance of sample

### 2.6.4. ABTS Radical Scavenging Activity

ABTS radical scavenging activity of lichen samples were made according to the method developed by Arnao et al. (2001). The absorbance was measured at 734 nm. BHT and rutin used as standards. The tests were carried out three times. The results are calculated from the following equation:

$$\% \text{ Activity: } \left[ \frac{A_0 - A_1}{A_0} \right] \times 100 \quad (2)$$

A<sub>0</sub>=Absorbance of control

A<sub>1</sub>= Absorbance of sample

### 2.6.5. Total Antioxidant Capacity

Total antioxidant capacity of the extracts was defined according to Prieto et al., (1999). Absorbance was measured at 695 nm. The results were calculated as µg ascorbic acid equivalent (AAE)/mL lichen sample from ascorbic acid

standard graphical equation. The tests were carried out three times.

### 2.6.6. DPPH Radical Scavenging Activity

The free radical scavenging activity of ethanol and acetone solvent extracts of *D. miniatum* and *X. conspersa* were measured by utilizing the method of Brand-Williams et al. (1995). Lichen extracts were prepared at 250-1000 µg/mL concentrations. The percentage inhibition was established by comparing the results of the test and the control. The tests were carried out three times. Percentage of activity was calculated using the following formula:

$$\% \text{ Activity: } \left[ \frac{A_0 - A_1}{A_0} \right] \times 100 \quad (3)$$

A<sub>0</sub>=Absorbance of control

A<sub>1</sub>= Absorbance of sample

## 4. Results and Discussion

### 4.1. Antibacterial Activity

The existence of biologically active compounds in lichens is studied in worldwide (Molnar and Farkas, 2010). The utilization of lichens in medicinally is based on their unique and biologically active substances, such as antimicrobial actions (Manojlovic et al., 2002; Saenz et al., 2006).

Results acquired in the present study demonstrated that the studied lichen extracts have possible antibacterial effect against all the test bacteria except for *S. enterica* and *E. coli*. Acetone extracts showed higher activity when compared to ethanol extracts (Table 3).

**Table 3.** Inhibition zones of the lichen extracts (mm)

Microorganisms	EED	AED	EEX	AEX	Tetra	Genta	DMSO
<i>E. coli</i>	NA	NA	NA	NA	18	17	NA
<i>Y. pseudotuberculosis</i>	NA	8	8	6	NA	18	NA
<i>B. cereus</i>	7	16	9	18	18	21	NA
<i>S. enterica</i>	NA	NA	NA	NA	17	18	NA
<i>B. subtilis</i>	12	11	12	18	12	18	NA
<i>P. vulgaris</i>	8	12	10	16	10	16	NA
<i>B. megaterium</i>	NA	7	14	NA	20	15	NA
<i>S. aureus</i> subsp. <i>aureus</i>	NA	6	9	11	22	14	NA
<i>K. pneumoniae</i>	NA	NA	8	6	15	19	NA
<i>G. rubripertincta</i>	NA	NA	10	15	17	14	NA
<i>E. aerogenes</i>	6	9	10	15	NA	20	NA

EED: Ethanol extract of *D. miniatum*; AED: Acetone extract of *D. miniatum*; EEX: Ethanol extract of *X. conspersa*; AEX: Acetone extract of *X. conspersa*; NA: No Activity; Tetra: Tetracycline 10µg/disc; Genta: Gentamycine 10µg/disc

The highest antibacterial activity obtained against *B. subtilis* (18 mm) and *B. cereus* (18 mm) and the lowest activity was found against *E. aerogenes* (6 mm), *S. aureus* (6 mm), *K. pneumoniae* (6 mm) and *Y. pseudotuberculosis* (6 mm). DMSO which was used as negative control exhibit no activity against bacteria. Tetracycline demonstrated higher effect than the lichen extracts. When antibacterial activity of the extracts was compared, it was found that *X. conspersa* extracts was more active than *D. miniatum*.

The antibacterial efficiency of the extracts of *X. conspersa* and *D. miniatum* are seen in Table 4. The results revealed that the lichen extracts exhibited antibacterial activities at variable degrees against test bacteria, with MIC values varying from 3.75 to 0.02929 mg/mL. The extracts of *X. conspersa* have lower MIC values than *D. miniatum*.

There are very limited studies about antimicrobial activity of *D. miniatum*. For example, Aslan et al. (2006) determined that methanol extract of *D. miniatum* weak antimicrobial activity. The extract showed no antifungal activity but the extract was active against *B. subtilis* and *Clavibacter michiganensis*. In agreement with this study we also found activity against *B. subtilis*. On the other hand, there are studies about antimicrobial activity of different *Dermatocarpon* species. Balasubramanian and Nirmala (2014a) evaluated antimicrobial activity of ethyl acetate, acetone,

chloroform, diethyl ether, methanol, ethanol and hexane extracts of *Dermatocarpon vellereum* Zschacke. Sharma et al. (2012) searched methanol and acetone extracts of *Dermatocarpon* spp. against five pathogenic clinical isolates of *E. coli*, and *S. aureus*. Results of these studies and our study are different because of collecting lichens different localities and using different *Dermatocarpon* species.

*X. conspersa* lichen was also studied by other researchers. For example, Duman (2009) investigated acetone extract of *X. conspersa* against *Escherichia coli*, *Enterococcus faecalis*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium* and *Pseudomonas aeruginosa*. Acetone extract of *X. conspersa* lichen was active against all the tested bacteria except for *S. aureus* and *P. aeruginosa*. *X. conspersa* lichen was effective against *Colletotrichum acutatum*, *C. coccodes* and *C. gloeosporioides* which cause anthracnose in red pepper (Jeon et al., 2009). Laska and Kiercul (2014) studied pharmacologic activity (antibacterial, antiviral and anticancer) of metabolites isolated from *X. conspersa* lichen. Acetone extract of *X. conspersa* lichen inhibited biofilm formation of *Candida albicans* (Millot et al., 2017). It was also reported that stictic acid which was obtained from *X. conspersa* lichen has antioxidant activity (De Paz et al., 2010). Studies of mentioned above and our study are different because of using different solvent.

**Table 4.** MIC values of the lichen extracts (mg/mL)

Bacteria	EED	AED	EEX	AEX
<i>E. coli</i>	NA	NA	NA	NA
<i>Y. pseudotuberculosis</i>	NA	3.75	3.75	3.75
<i>B. cereus</i>	3.75	0.234375	0.46875	0.05859
<i>S. enterica</i>	NA	NA	NA	NA
<i>B. subtilis</i>	3.75	0.1171875	0.1171875	0.05859
<i>P. vulgaris</i>	3.75	0.1171875	0.05859	0.02929
<i>B. megaterium</i>	NA	1.875	0.46875	NA
<i>E. aerogenes</i>	15	3.75	1.875	0.1171875
<i>S. aureus</i> subsp. <i>aureus</i>	NA	3.75	0.46875	0.1171875
<i>K. pneumoniae</i>	NA	-	3.75	1.875
<i>G. rubripertincta</i>	NA	NA	0.46875	0.234375

NA: No Activity

#### 4.2. Antioxidant Activity

Ethanol and acetone extracts of *X. conspersa* and *D. miniatum* collected from Black Sea Region were screened with regard to their contents of total phenols. Table 5 is seen the total phenol contents that were determined by Folin Ciocalteu

reagent in terms of gallic acid equivalent (GAE). Total phenolic content of the extracts are ranged from 25.08±0.002 to 209.92±0.01 µg GAE/mL lichen extract. The highest and the lowest phenolic content were measured in acetone extract of *X. conspersa* and acetone extract of *D. miniatum*, separately. Phenolic compounds have



been declared to be linked with antioxidative effect in biological systems, acting as scavengers of singlet oxygen and free radicals (El Hajaji et al., 2010).

Flavonoids are one class of secondary plant metabolites that are recognised as Vitamin P. They are commonly utilized in plants to generate yellow and other coloured pigments which have a crucial role in the colors of plants. Moreover, flavonoids exhibit significant anti-cancer, anti-inflammatory and anti-allergic activities (Rebeya et al., 2014).

The amount of total flavonoid content is expressed as  $\mu\text{g CE/mL}$  lichen extract. Total flavonoid content of the lichens was given in Table 5. Acetone extract of *X. conspersa*

exhibited maximum and ethanol extract of *D. miniatum* showed minimum flavonoid content. *X. conspersa* lichen possesses higher total flavonoid activity than *D. miniatum* lichen.

The phosphomolybdenum procedure is primarily based on the reduction of molybdenum, Mo (VI) to Mo (V) by the effect of antioxidant substances and the generation of a green phosphate, Mo (V) complex with a highest absorption at 695 nm (Hossain et al., 2017). Total antioxidant capacities of the extracts are given in Table 5. Total antioxidant capacity of the extracts increased in the following order: Ethanol extract of *D. miniatum* > acetone extract of *X. conspersa* > ethanol extract of *X. conspersa* > acetone extract of *D. miniatum*.

**Table 5.** Total phenolic and flavonoid contents and total antioxidant capacity of the tested lichen extracts

Lichen	Total phenolic content ( $\mu\text{g GAE/mL}$ lichen extract)	Total flavonoid content ( $\mu\text{g CE/mL}$ lichen extract)	Total antioxidant capacity ( $\mu\text{g AAE/mL}$ lichen extract)
Ethanol extract of <i>D. miniatum</i>	86.27 $\pm$ 0.007	44.79 $\pm$ 0.006	92.59 $\pm$ 0.02
Acetone extract of <i>D. miniatum</i>	25.08 $\pm$ 0.002	52.20 $\pm$ 0.01	48.43 $\pm$ 0.07
Ethanol extract of <i>X. conspersa</i>	67.72 $\pm$ 0.01	68.68 $\pm$ 0.03	52.58 $\pm$ 0.05
Acetone extract of <i>X. conspersa</i>	209.92 $\pm$ 0.01	83.60 $\pm$ 0.02	63.54 $\pm$ 0.006

Values are expressed as means of three replicates  $\pm$  SD

DPPH radical scavenging activity of the extracts was indicated in Table 6. DPPH radical quenching activity of the studied lichen extracts increase as ethanol extract of *X. conspersa* > acetone extract of *D. miniatum* > ethanol extract of *D. miniatum* > acetone extract of *X. conspersa*. In addition, DPPH radical scavenging activity of extracts is a dose-dependent manner.

ABTS radical scavenging activities of the extracts are given in Table 6. The highest activity was detected in ethanol extract of *X. conspersa*. ABTS radical scavenging activity of extracts is a dose-dependent manner.

ABTS and DPPH radicals scavenging activities of the extracts and standards also are expressed as half maximal inhibitory concentrations ( $\text{SC}_{50}$ ) values, calculated from the regression equations prepared from the concentrations of samples. A

higher scavenging activity is associated with a lower  $\text{SC}_{50}$  value.

Chelating agents can inhibit radical formations by stabilizing transition metals consequently reducing free radical injury. Moreover, some phenolic compounds demonstrate antioxidant action through the chelating of metal ions (Yumrutas and Saygideger, 2012). Metal chelating activity of the lichens is shown in Table 6. Only extracts of *X. conspersa* exhibited too weak metal chelating activity. EDTA which is standard antioxidant showed very high activity when compared with the extracts.

There are some studies about antioxidant activity of *Dermatocarpon* and *X. conspersa* lichen species. For example, Aslan et al., (2006) was stated that  $\text{IC}_{50}$  values of the methanol extract of *D. miniatum* was found as 396.1  $\mu\text{g/mL}$  and total phenolic content was 2.9 %.

**Table 6.** DPPH and ABTS radical scavenging activities and metal chelating activity of the tested lichen extracts

Lichen	Concentration (µg/mL)	DPPH radical scavenging activity (% activity)	SC <sub>50</sub> values for DPPH radical scavenging activity	Metal chelating activity (% activity)	ABTS radical scavenging activity (% activity)	SC <sub>50</sub> values for ABTS radical scavenging activity
Ethanol extract of <i>D. miniatum</i>	250	6.87±0.01	939.34±8.73	NA	21.14±0.05	531.33±14.24
	500	22.72±0.03		NA	59.21±0.008	
	750	34.17±0.04		NA	77.20±0.010	
	1000	41.63±0.03		NA	83.87±0.02	
Acetone extract of <i>D. miniatum</i>	250	10.43±0.02	1072.66±26.03	NA	8.73±0.03	633.87±0.64
	500	16.49±0.02		NA	38.36±0.04	
	750	35.20±0.02		NA	63.41±0.02	
	1000	49.03±0.01		NA	81.12±0.02	
Ethanol extract of <i>X. conspersa</i>	250	44.34±0.01	614.68±1.95	0.345±0.005	85.48±0.03	209.00±5.55
	500	50.22±0.06		0.522±0.02	93.39±0.002	
	750	57.92±0.02		1.27±0.01	94.07±0.006	
	1000	64.92±0.005		4.11±0.02	96.84±0.007	
Acetone extract of <i>X. conspersa</i>	250	18.84±0.01	1350±34.32	NA	NA	NA
	500	24.61±0.03		NA	NA	
	750	29.20±0.03		NA	NA	
	1000	32.25±0.01		5.71±0.02	24.36±0.007	
BHT	250	88.85±0.01	204.25±2.62	NS	93.48±0.01	170.1±0.80
	500	89.55±0.005		NS	93.92±0.006	
	750	90.27±0.01		NS	94.43±0.004	
	1000	91.55±0.008		NS	96.65±0.008	
Rutin	250	86.80±0.008	209.13±5.77	NS	78.54±0.04	272±8.93
	500	87.91±0.003		NS	81.94±0.01	
	750	90.60±0.004		NS	85.26±0.01	
	1000	91.89±0.01		NS	87.63±0.006	
EDTA	250	NS	NS	94.27±0.03	NS	NS
	500	NS	NS	97.53±0.01	NS	NS
	750	NS	NS	99.89±0.0004	NS	NS
	1000	NS	NS	99.91±0.0004	NS	NS

NA: No Activity; NS: No Studied. Values are expressed as means of three replicates ± SD

On the other hand, we found total phenolic contents of ethanol and acetone extracts of *D. miniatum* as 86.27±0.007 µg GAE/mL and 25.08±0.002 µg GAE/mL, respectively in the study. Total antioxidant activity, total phenolic content and reducing power of methanol and water extracts of *Dermatocarpon intestiniformis* was also screened (Odabasoglu et al., 2005). Balasubramanian and Nirmala (2014b) investigated antioxidant properties of *Dermatocarpon vellereum* lichen.

Kumar et al. (2014) explained that methanol extract of *X. conspersa* exhibited high antioxidant activity. Similarly, Sökmen et al. (2018) found high CUPRAC activity in acetonitrile extracts of *X. stenophylla* lichen. We also found antioxidant activity in *X. conspersa* extracts at varying degrees.

## Conclusion

The results of the current research proposed that *D. miniatum* and *X. conspersa* lichens might be a possible natural alternative of synthetic antioxidants and might have gained significance as healing agent in hindering or decelerating oxidative stress linked with degenerative diseases. Furthermore, these lichens might be utilized to produce brand, different and more effective antimicrobial medicines of natural origin in curing infectious illnesses. Detailed studies are needed to determine the biologically effective compounds of *D. miniatum* and *X. conspersa* lichens.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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