



## The free radical scavenging activities of biochemical compounds of *Dicranum scoparium* and *Porella platyphylla*

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### Abstract

The bryophytes studies carried out in our country are mainly for bryofloristic purposes and the studies on biochemical contents are very limited. *Dicranum scoparium* and *Porella platyphylla* taxa of bryophytes were used in the present study carried out to determine the free radical scavenging activities, fatty acid, and vitamin contents. In this study, it was aimed to underline the importance of bryophytes for scientific literature and to provide a basis for further studies on this subject. The data obtained in this study indicate that the DPPH radical scavenging effect of *D. scoparium* taxon is significantly higher than that of *P. platyphylla* taxon. It is known that there is a strong relationship between the phenolic compound content of methanol extracts of the plants and the DPPH radical scavenging efficiency. When the fatty acid contents were examined, it was observed that levels of all unsaturated fatty acids were higher in the *P. platyphylla* taxon than the *D. scoparium* taxon, except for  $\alpha$ -Linolenic acid. When the vitamin contents of species were compared, it was determined that D-3,  $\alpha$ -tocopherol, stigmasterol, betasterol amount was higher in *Dicranum* taxon.

**Keywords:** DPPH, Fatty Acid, Vitamin, Dicranaceae, Porellaceae

### *Dicranum scoparium* ve *Porella platyphylla* taxonlarının biyokimyasal bileşiklerinin serbest radikal temizleme faaliyetleri

#### Öz

Ülkemizde briyofitler ile ilgili olan çalışmalar genellikle briyofloristik amaçlı olup serbest radikal temizleme aktiviteleri ve yağ asidi içerikleri gibi diğer amaçlı çalışmalar yok denecek kadar azdır. Serbest radikal temizleme aktiviteleri ve yağ asidi içeriklerini belirlemek adına yaptığımız bu çalışmada, briyofitlere ait *Dicranum scoparium* ve *Porella platyphylla* taksonları kullanılmıştır. Bu çalışmada briyofitlerin bilim dünyasındaki önemine bir ivme kazandırmak ve bu konuda ileride yapılacak diğer çalışmalara temel oluşturulması amaçlanmıştır. Çalışmamızda elde edilen verilerde *Dicranum scoparium* türünün DPPH radikal temizleme etkisinin *P. platyphylla* türüne göre belirgin düzeyde fazla olduğu gözlenmiştir. Bitki ekstraktları içeriğindeki polifenolik bileşiklerin düzeyi ile DPPH radikalini temizleme etkinliği arasında güçlü bir ilişki bulunduğu bilinmektedir. Yağ asidi içerikleri incelendiğinde ise  $\alpha$ -Linolenik asit dışındaki bütün yağ asitlerinin *P. platyphylla* türünde *D. scoparium* türüne göre fazla olduğu gözlenmiştir. Vitamin içerikleri karşılaştırıldığında, *Porella* taksonuna göre *Dicranum* taksonunda D-3, a-tokoferol, stigmasterol, betasterol miktarının daha yüksek olduğu tespit edildi.

**Anahtar kelimeler:** DPPH, Yağ asidi, Vitamin, Dicranaceae, Porellaceae.

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## 1. Introduction

Bryophytes are the second largest group in the plant kingdom with about 25.000 bryophyte species and they can be found in any ecosystem (Glime, 2007; Asakawa et al., 2013a). Compared to higher plants, the use of bryophyte for human consumption can be neglected due to its low-calorie values (Forman, 1968) and poor organoleptic properties. Traditionally, the use of bryophytes as a food source is limited for periods of famine, but in the northern regions of Europe and America, bryophytes are used as bread or soup ingredients. Around the pole, bryophytes are widely used as animal feed (Klavina, 2015).

*Dicranum scoparium* is a plant belonging to Bryophyta taxon of Dicranaceae family. Preferring the arctic and cold climates, it is one of 13 taxa of *Dicranum* in our country (Kürschner and Frey, 2011). *Porella platyphylla* is a plant belonging to Marchantiophyta filum of Jungermanniopsida class of Porellales ordo of Porellaceae family. It is one of 6 taxa of *Porella* species (Özenoğlu et al., 2009). *Dicranum scoparium* is the taxon that prefers the shadowy and semi-arid acidic environments, while *Porella platyphylla* is the taxon that generally prefers shadowy and moist environments.

Bryofites are widely used as medicinal plants in ethnopharmacology and in the treatment of wounds and burns due to their large content of biologically active compounds. More specifically, bryophytes show antibacterial, antifungal, antiviral activity, antioxide, antiplatelet, antithrombin, insecticidal, neuroprotective activities and cytotoxicity against cancer cells. (Cheng et al., 2012). Besides, bryophytes are an interesting group of plants that attract the attention of chemists and bryologists, due to the diversity of biologically active compounds they contain, including their lipid content. (Zinsmeister and Mues, 1990; Whittemore, 1991; Dembitsky, 1993). Fatty acids, which are abundant in bryophytes, are common in most of the organisms. However, many bryophytes have typically acetylene (Anderson et al., 1974; Jamieson, 1975; Jamieson and Reid, 1976a; Kohn, 1987a; Kohn, 1987b; Dembitsky et al., 1993b; Dembitsky et al., 1993c; Dembitsky and Rezanka, 1994;), arachidonic and eicosapentaenoic acid at high amounts. Such compounds are not found in the rest of the plants (Gellerman, et al., 1972; Hartmann, et al., 1986; Hansen and Rossi, 1991; Beike et al., 2014; Shanab et al., 2018; Lu et al., 2019).

In this study, it was aimed to emphasize the importance of bryophytes in the scientific literature and to determine some biochemical activities contents (fatty acid, vitamin, and DPPH) of *Dicranum scoparium* and *Porella platyphylla* taxa and form the basis for future studies.

## 2. Materials and Methods

### 2.1. Herbal extract:

The plants used in this study were collected from Kamilet Valley (Artvin-Arhavi). Our samples were weighed in 1g and then centrifuged after adding 10ml (80%) methanol and digested with homogenizer (1 min). After shredding, all samples were centrifuged at 6000 rpm at +4 ° C. At the end of the centrifuge, the solvent of the supernatant portion was removed using rotovapor. It was then dissolved in 10 ml of methanol and kept until use at -20 °C. DPPH analysis was done from methanol extract. The same procedure was performed with hexane isopropanol (hexane instead of methanol) for fatty acid and vitamin content analysis. Herbal extract of the samples were done by revising the method of Aydın et al. (2011).

### 2.2. Free radical (DPPH) scavenging activity

The free radical scavenging effect of Bryophytes extracts was assessed by the discoloration of a methanolic solution of DPPH• (Brand-Williams et al., 1995). DPPH ( $\alpha$ -Diphenyl- $\beta$ -picrylhydrazyl) prepared in methanol was used as a free radical. 4 ml of DPPH solution were added to the test tubes, respectively. Then 50, 100, 250, 500, 1000,  $\mu$ L. plant extracts were added and mixed with vortex. It was incubated for 30 minutes at room temperature in the dark and at the end of the incubation the absorbance of the mixture was measured at 517 nm in a spectrophotometer (Hsu et al., 2006). Vanillic acid was used as a positive control group. Vanillic acid (100 mg) was prepared by dissolving in 10 mL of dimethyl sulfoxide (DMSO) (Özcan et al., 2019). Vanillic acid is a phenolic compound with known antioxidant effect (Dianat et al., 2016; Anbalagan et al., 2017; Özcan et al., 2019).

Decreased absorbance, remaining DPPH amount were determined as free radical scavenging activity. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control – Abs sample) / (Abs control)]  $\times$  100.

Where, Abs control is the absorbance of DPPH radical + methanol and Abs sample is the absorbance of DPPH radical + sample extract /standard (methanol).

### 2.3. Extraction of fatty acids and gas chromatographic analysis of fatty acid methyl esters

The lipid extractions of samples were performed using Hara and Radin (1978) method, in which 3:2 (v/v) hexane isopropanol mixture was used. The hexane phase was taken into separate tubes and 5 ml 2% methanolic sulfuric acid was added onto it; the mixture was left at 55 °C for 12 hours. Then, 5 ml of 5% sodium chloride was added and the fatty acid methyl esters were extracted with 5 ml of n-hexane. The mixture was treated with 5 ml of 2% KHCO<sub>3</sub> solution, then the n-hexane phase was vaporized with nitrogen stream (Christie, 1992), fatty acid methyl ester residues were dissolved in 1 ml hegzane and taken to autosampler vials. Then, mixtures of fatty acid methyl esters of the samples were analyzed.

Fatty acid methyl esters were analyzed by SHIMADZU GC 17 gas chromatography and SP™ -2380 capillary GC arm (L × I.D. 30 m × 0.25 mm, df 0.20 µm) (Supelco, Sigma, USA) was used for this analysis. During the analysis, before the analysis of the fatty acid methyl esters of the samples, the mixtures of the standard fatty acid methyl esters were injected, the retention times of each fatty acid were determined, and then the necessary programming was done, and the analysis of the mixtures of the fatty acid methyl esters of the samples was performed.

### 2.4. Analysis of A, D, E, K vitamins and phytosterols

Vitamin and phytosterol analysis of the samples was done by revising the method of Aydın et al. (2011). Samples were weighed in 1g and homogenized with n-hexane/isopropyl at 3/2 (v/v) ratio and after the hydrolysis with 5% KOH at 85 °C (15 min), the tubes were removed from the oven and cooled at room temperature, 5 ml of distilled water was added and mixed well. After this process, the mixture in the tubes was separated into phases and the upper hexane phase was taken into clean centrifuge tubes and its solvent was evaporated. Then, the Acetonitrile / methanol mixture (50% + 50%, v/v) was prepared, and 1 ml was dissolved in this mixture, taken into autosames and prepared for analysis.

Acetonitrile / methanol (60% + 40%, v/v) mixture was used as mobile phase. Mobile phase flow rate was determined as 1 ml/min. DAD-UV detector was used for analysis. Supelcosil™ LC 18 (15 x 4.6 cm, 5 µm; Sigma, USA) column was

used as the colon. Detection wavelength 326 nm for vitamin A, 202 nm for vitamins E, D, K and phytosterols.

### 2.5. Statistical analysis

Statistical analysis was performed using SPSS 2018 software (ver 18.0). The experimental results were reported as mean ± SEM (standard error of means). Analysis of variance (ANOVA) and an LSD (least significant difference) test were used to compare the experimental groups with the control (vanillic acid).

## 3. Results

At the end of the present study, some of the saturated and unsaturated fatty acid and vitamin contents of bryophytes extracts and the free radical scavenging activities (DPPH) were determined (Table 1, Table 2 and Table 3). Compared to vanilic acid, *Dicranum* taxon had higher DPPH scavenging effect than *Porella* (P<0.001) (Table 1 and Figure 4).

### 3.1. DPPH values

It was observed that the DPPH radical scavenging effect in *Dicranum* type and *Porella* type increased as the amount of extract increased (Figure 1, 2, 3). Compared with vanillin, DPPH free radical scavenging effect was observed in 50, 250, 500, and 1000 ml of *Dicranum* taxon compared to *Porella* taxon (P<0.001) (Figure 4 and Table 1).

### 3.2. Fatty acids contents

At the end of this study, it was observed while determining the fatty acid levels that all of the fatty acids except for α-Linolenic acid (18:3 n3) were at higher levels in *Porella platyphylla* taxon (P<0.001). Especially Palmitic acid (16: 0), Linoleic acid (18: 2, n-6c), -eicosatrienoic acid (20: 3), and Stearic acid (18: 0) were found to be significantly difference in between two taxon. Comparing these fatty acids between the two taxon, it was found that they were significantly more in the *Porella* type (P<0.001) (Table 2).

### 3.3. Vitamin contents

It was determined that the amounts of vitamin D-3, α-tocopherol, stigmasterol, betasterol were higher in *Dicranum* taxon than in *Porella* (p<0.001). These sterols are compounds with known antioxidant effects. However, it was observed that the concentration of ergosterol and cholesterol was higher in the *Porella* taxon (p<0.001) (Table 3).

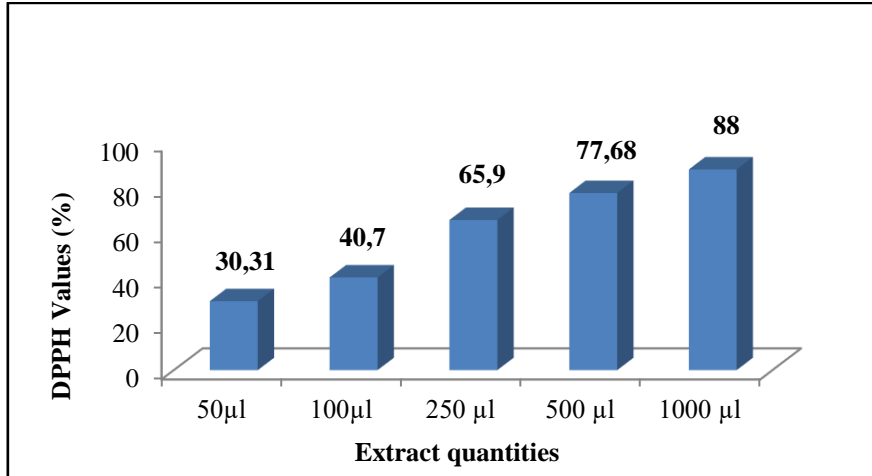


Figure 1. DPPH values of vanillic acid

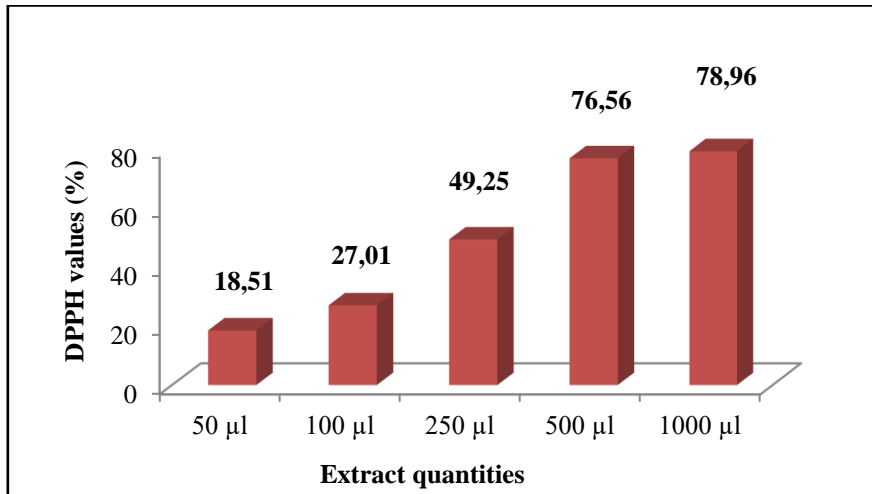


Figure 2. DPPH values of *Dicranum scoparium*

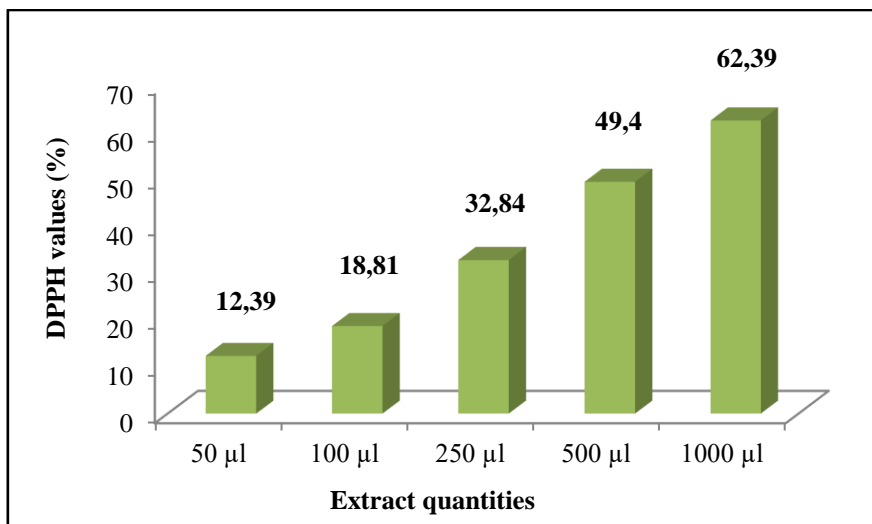


Figure 3. DPPH values of *Porella platyphylla*

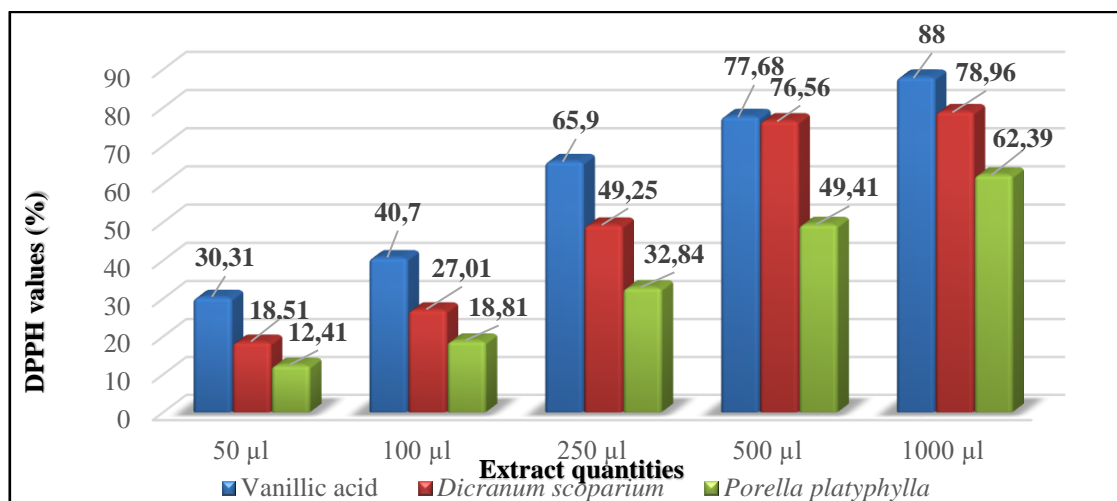


Figure 4. Comparison of DPPH values of all extract quantities

Table 1. DPPH values of all extract groups

Extract quantities	DPPH values (%)		
	Vanillic acid	<i>Dicranum scoparium</i>	<i>Porella platyphylla</i>
50 µl	30.31±0.12	18.51±0.3 <sub>1<sup>d</sup></sub>	12.41±0.02 <sub>d</sub>
100 µl	40.70±0.08	27.01±0.0 <sub>3<sup>d</sup></sub>	18.81±0.01 <sub>d</sub>
250 µl	65.90±0.70	49.25±0.0 <sub>1<sup>d</sup></sub>	32.84±0.01 <sub>d</sub>
500 µl	77.68±0.10	76.56±0.0 <sub>1<sup>d</sup></sub>	49.41±0.01 <sub>d</sub>
1000 µl	88.00±0.20	78.96±0.0 <sub>1<sup>d</sup></sub>	62.39±0.01 <sub>d</sub>

d: p<0.001, c: p<0.01, b: p<0.05, a: p>0.05

Table 2. Fatty acid contents of *D. scoparium* and *P. platyphylla* species (µg/g)

Fatty acids	Quantities of fatty acid (µg/g)	
	<i>Dicranum scoparium</i>	<i>Porella platyphylla</i>
<b>SAFA(Saturated)</b>		
14:0 (Myristic acid)	0.35±0.06	0.36±0.01 <sup>a</sup>
16:0 (Palmitic acid)	5.62±0.54	17.24±0.29 <sup>d</sup>
18:0 (Stearic acid)	1.75±0.18	4.64±0.57 <sup>d</sup>
<b>MUFA (Monounsaturated)</b>		
16:1, n-7 (Palmitoleic acid)	0.32±0.03	1.16±0.14 <sup>d</sup>
<b>PUFA (Polyunsaturated)</b>		
18:2, n-6c (Linoleic acid)	2.21±0.14	22.22±1.06 <sup>d</sup>
18:3 n6 (α-Linolenic acid)	0.25±0.03	0.46±0.06 <sup>c</sup>
18:3 n3 (α-Linolenic acid)	3.61±0.19	2.39±0.17 <sup>d</sup>
20:3 (-eicosatrienoic acid)	2.29±0.20	11.40±0.50 <sup>d</sup>
20:5 (Eicosapentaenoic acid)	0.35±0.04	2.27±0.22 <sup>d</sup>

d: p<0.001, c: p<0.01, b: p<0.05, a: p>0.05

Table 3. Lipophilic vitamin and phytosterol contents of *D. scoparium* and *P. platyphylla* species (µg/g)

ADEK vitamins	<i>Dicranum scoparium</i>	<i>Porella platyphylla</i>
D-3	0.30±0.001 <sup>d</sup>	0.10±0.001
α-Tocopherol (Vitamin E)	0.47±0.01 <sup>d</sup>	0.27±0.10
Retinol (Vitamin A)	0.02±0.001	0.02±0.002
<b>Phytosterols</b>		
Ergosterol	0.02±0.01	0.07±0.001 <sup>b</sup>
Stigmasterol	7.88±0.02 <sup>d</sup>	3.24±0.06
Betasterol	1.34±0.12 <sup>d</sup>	0.2±0.001

d: p<0.001, c: p<0.01, b: p<0.05, a: p>0.05

#### 4. Discussion and Conclusion

The bryophytes are used for various purposes in many cultures. The reason for this is the various materials they contain (terpenoids, simple benzoic, cinnamic, and phthalic acid derivatives, coumarins, and some nitrogen-containing aromatic compounds, the benzonaphthoxanthones) the chemical contents of bryophytes may vary significantly depending on their types and they may show a wide diversity. Some of the reasons for this diversity are the habitat, seasonal changes, level of water and moisture exposure, and materials taken from the environment (Heinrichs, 2000).

In a study carried out by Chobot et al., (2006), the antioxidant activities of 5 bryophytes were examined. In that study, the relationship between phenolic content and antioxidant effect of bryophytes was investigated in vitro. The species used in that study were *Ceratodon purpureus* (Hedw.) Brid. (*Dicranaceae*), *Dicranum polysetum* Sw. *Dicranum scoparium* Hedw. (*Dicranaceae*), *Leucobryum glaucum* (Hedw.) Angstr. (*Leucobryaceae*), and *Mnium*

*marginatum* (With.) P. Beauv. (Mniaceae). As a result of the study, it was determined that *C. purpureus* contains lipids and flavonoids, *D. polysetum* has lipids and acetylenic acid, *D. scaparium* has lipids, acetylenic acid, and flavonoids, *L. glaucum* has sterols, and *M. marginatum* has terpenes. Folin-Ciocalteu reactive was used for determining the relationship between antioxidant activity and phenolic content, and caffeic acid was used as a positive control. At the end of the study, it was observed that all the species had lower antioxidant activity levels when compared to the caffeic acid. However, although lower than caffeic acid, they were found to have antioxidant activity. At the end of this study, it was determined that the effects of bryophyte extracts investigated here had no significant relationship with the total phenolic content. However, in previous studies, it was emphasized that the synergetic or antagonistic effects of various contents might alter the antioxidant activity (Nishiki et al., 2007).

In a similar study, using the DPPH radical scavenging assay, the bioactivity-guided fractionation of *Plagiochila Ovalifolia* ether extract resulted in the isolation of antioxidative 3,5-dihydroxy-2-(3-methyl-2-butenyl)-bibenzyl and plagiochin D (Sadamori, 2009). At the same time,  $\alpha$ -tocopherol found in all liverworts predicted that these plants could provide antioxidants for fat bodies. Similarly, it was also observed in the present study that *Dicranum* containing  $\alpha$ -tocopherol, which shows antioxidant effect, exhibited significant DPPH effect.

It was reported in previous studies that the DPPH radical scavenging effect is related with the amount of phenolic content in the extracts. In other words, there is a strong relationship between the level of phenolic compounds in the herbal extracts and the scavenging efficiency of DPPH (Sadamori, 2009; Dey and De, 2012). In the present study, a high level of DPPH scavenging activity of *Dicranum* taxa suggested that *Dicranum* taxa are richer in terms of vitamin and phytosterol contents (vitamin D-3 level,  $\alpha$ -tocopherol, stigmasterol, betasterol) (Table 2).

Anderson et al. (1974), Karunen (1982), and Sewon (1992) used the distribution of fatty acids as a criterion for the classification of bryophytes. These researchers reported that there were differences in bryophytes and liverwort families in terms of fatty acid composition and these differences are at higher levels among sub- and super-groups. For instance, they considered the

presence or absence of several fatty acids such as acetylenic acid and certain polyunsaturated acids as an indicator.

Several compounds identified in the previous studies, which have been published to date, are 6a, 9-18:2 for *Riccia fluitans* (Kohn, 1987b) and *Fontinalis antipyretica* (Jamieson and Reid, 1976b) and 6a-18: 1, 9a-18:1, 12a-18: 1, 6a, 9,12-18: 3, 6a, 9,12,15-18: 4, 8a, 11,14-20: 3, 5a, 8,11,14-20: 4 for other bryophytes (Dembitsky, 1993; Dembitsky et al., 1993c).

In the previous studies, it was determined that the bryophytes yielded much lower acetylenic acid levels (0% and 3.7) and the main fatty acids contained in species such as *Bryum bicolor*, *Hylocomium splendens*, *Mnium cuspidatum* Hedw., *Mnium*, *Plagiothecium laetum*, and *Pleurozium schreberi* were reported to be 20: 4 and 20: 5 acids (Gellerman et al., 1975; Karunen, 1982; Al-Hasan et al., 1989). Hansen and Rossi (1991) investigated 25 bryophytes for 20: 4 and 20: 5 fatty acids. They reported that 6 species from Rhytidiaceae and Hypnaceae families had the highest levels (23-47%) of these polyunsaturated acids.

In the studies carried out to date on Bryophyta species, significant differences were observed in both C16 and C18 polyunsaturated acids (angiosperm types) and C20 polyunsaturated fatty acids (bryophytes type) acids. The similarity of the alignment of 16: 3 (n-3) and 18: 3 (n-3) fatty acids, which are at a high level in Bryophyta, to the composition of angiosperms suggested that this similarity is an indicator of a high level of evolution (Karunen, 1982; Dembitsky, 1993). Similar to these studies, given the increases in fatty acid levels in the present study, it was observed that 18:3 n3 fatty acid was at a significant level in *Dicranum* species and other fatty acids in *Porella* species (d: p<0.001). The high level of 18:3 (n-3) fatty acid, which is considered to be the indicator of high evolutionary level, in *Dicranum* species suggested that it is related with a high level of DPPH effect in this species.

In the present study, as shown in the previous studies, it was determined that the diversity of fatty acid contents is not solely sufficient for antioxidant effects such as radical scavenging and that the synergic effect of other antioxidant components (such as vitamin, sterol, and phenolics) in the herbal extract has contribution. We believe that the changes in fatty acids originate from the variabilities in nutritional

differences in habitats of bryophytes, because the difference in carbon source in use affects the activity of enzymes, which are responsible for the synthesis of these fatty acids, might result in differences in amounts of fatty acids and antioxidants as  $\alpha$ -tocopherol, stigmasterol, setasterol. By carrying out more studies on the antioxidant contents of bryophytes, these contents might be used in pharmacology and food industries in future.

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