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Determination of Total Phenol Contents, Antibacterial and Antioxidant Activity of Some Mosses Species

Bazı Yosun Türlerinin Toplam Fenol İçeriklerinin, Antibakteriyel ve Antioksidan Aktivitelerinin Belirlenmesi

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Abstract

The aim of this study were to determine total phenol contents, antibacterial and antioxidant activity of bryophyte species including *Plasteurhynchium striatum (Spruce) M. Fleisch, Palamocladium euchloron (Bruch ex Müll. Hal.) Wijk & Margad.* and Cratoneuron filicinum (Hedw.) Spruce and Campyliadelphus chrysophylus (Brid.) R.S. Chopra. Antibacterial activity of methanolic, ethanolic, chloroform, acetone and water extracts of these species was determined by microdilution method. Antioxidant activity of these species was evaluated by 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging ability. The total phenol content of these species was determined quantitatively using the folin ciocalteu reagent, with gallic acid equivalents as the standard.

The highest DPPH free radical scavenging activity was observed in about 65% *C. filicinum*. Total phenolic content of *P. euchloron* was determined the highest extract value of 0,027±0,002 mg/g. The methanol extract of bryophytes was found to have significant antioxidant activity and phenolic contents. Antibacterial effect of the extracts was not observed against the tested microorganisms.

Keywords: Antimicrobial activity, Antioxidant activity, Bryophytes, Total phenol contents

Öz

Bu çalışmanın amacı *Cratoneuron filicinum (Hedw.)* Ladin, *Campyliadelphus chrysophylus* (Brid.) R.S. Chopra. *Plasteurhynchium striatum (Spruce) M. Fleisch, Palamocladium euchloron (Bruch ex Müll. Hal.) Wijk & Margad* gibi briyofit türlerinin toplam fenol içeriklerini, antibakteriyel ve antioksidan aktivitelerini belirlemektir. Bu türlerin metanolik, etanolik, kloroform, aseton ve su ekstraktlarının antibakteriyel aktiviteleri mikrodilüsyon yöntemi ile, antioksidan aktivitesi 2,2-difenil-1-pikrilhidrazil serbest radikal süpürme yeteneği ile, Toplam fenol içerikleri ise standart olarak gallik asit eşdeğerleri ile folin ciocalteu reaktifi kullanılarak nicel olarak belirlenmiştir.

En yüksek DPPH serbest radikal süpürme aktivitesi yaklaşık %65 ile C. filicinum'da gözlendi. P. euchloron'un toplam fenolik içeriği en yüksek ekstre değeri 0,027±0,002 mg/g olarak belirlenmiştir. Briyofitlerin metanol ekstraktının önemli antioksidan aktiviteye ve fenolik içeriğe sahip olduğu bulundu. Ekstraktların test edilen mikroorganizmalara karşı antibakteriyel etkisi gözlenmedi.

Anahtar Kelimeler: Antimikrobiyal aktivite, Antioksidan aktivite, Briyofitler, Toplam fenol içeriği

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1. Introduction

There has been an increasing interest in studies on the potential use of plants as natural biologically active compounds in the pharmaceutical industry all over the world (Pejin and Bogdanović-Pristov 2012, Pejin et al. 2013, Yağlıoğlu et al. 2017). The evaluation of the biological activities of the bryophytes provides a significant contribution to the search of possible drug sources.

Bryophytes, the second largest group, which comes after seed plants, were separated into three divisions within the subkingdom of Bryobiotina as a result of recent molecular studies. These divisions are composed of Bryophyta, Marchantiophyta, and Anthocerotophyta (Glime 2007, Goffinet and Shaw 2009).

Bryophytes has been widely used as a herbal medicine in China and India and among Native Americans since ancient times. Bryophytes have high amounts of terpenoids, phenolics, glycosides, fatty acids and some rare aromatic compounds that can contribute to the prevention of cancer and other chronic diseases (Mainasara et al. 2021).

Most of studies, however, have focused on higher vascular plants, specifically the angiosperms. Compared to angiosperms, pharmaceutical studies on bryophytes are relatively fewer (Magtoto et al. 2015). Similar to other plants, bryophytes possess antimicrobial and antioxidant properties (Bodade et al. 2008). Some research has indicated the potential of mosses as natural sources of antioxidant (Pejin and Bogdanović-Pristov 2012, Pejin et al. 2013, Yağlıoğlu et.al. 2017).

Extracts of some bryophytes plants that have a high concentration of phenolics have good antioxidant activity (Hinneburg et al. 2006, Rice-Evans et al. 1995). The antioxidant and total phenol content of *Marchantia diptera* Nees & Mont. (Marchantiaceae), *Ceratodon purpureus* (Hedw.) Brid. (Ditrichaceae), *Dicranum polysetum* Sw., *Dicranum scoparium* Hedw. (Dicranaceae), *Leucobryum glaucum* (Hedw.) Ångstr. (Leucobryaceae), *Mnium marginatum* (With.) P. Beauv. (Mniaceae), *Atrichum undulatum* (Hedw.) P. Beauv., *Polytrichum formosum* Hedw. (Polytrichaceae), *Pleurozium schreberi* (Willd. ex Brid.) Mitt. (Entodontaceae), *Thuidium tamariscinum* (Hedw.) Schimp. (Thuidiaceae), *Brachythecium rutabulum* (Hedw.) Schimp. (Brachytheciaceae), *Calliergonella cuspidata* (Hedw.) Loeske (Hypnaceae), *Hypnum mammillatum* (Brid.) Loeske (Hypnaceae), *Polytrichastrum alpinum* (Hedw.) G. L. Sm., *Bryum moravicum* Podp. and *Rhodobryum ontariense* (Kindb.) Kindb. medicinal bryophyte species have been studied by different researchers indicating that they have high antioxidant activity and phenolic content, which might give new direction in medicine as a source of antioxidant agents in the future (Hsiao et al. 1996, Chobota et al. 2008, Bhattarai et al. 2009, Cvetić et al. 2009, Pejin and Bogdanović-Pristov 2012, Pejin et al. 2013, Pejin et al. 2014).

Also, some researchers have performed phenolic content and antimicrobial activity of the Bryophytes (Cansu et al. 2013, Cansu et al. 2014, Tosun et al. 2014, Tosun et al. 2015, Çöteli et al. 2017, Çöteli et al. 2019).

The aim of this study were to determine the antioxidant and antimicrobial activity together with the total phenol content of four moss species including *Plasteurhynchium striatum* (Spruce) M. Fleisch, *Palamocladium euchloron* (Bruch ex Müll. Hal.) Wijk & Margad. *Cratoneuron filicinum* (Hedw.) Spruce, *Campyliadelphus chrysophylus* (Brid.) R.S. Chopra. that are also used as components of traditional medicine preparations. (Cui et al. 2005, Shai et al. 2010).

2. Material and Methods

2.1. Plant Material and Sample Preparation

Research materials were collectedfrom Zonguldak province in Turkey. They were identified and deposited in Zonguldak Bülent Ecevit University-Bryophyte herbarium (ZNG 2428, 3294, 5851, 5869). In this study, spectrophotometric measurements were measured with Tetra Mark T80 + UV / VIS Spectrometer PG Instruments. DPPH, BHT, BHA, gallic acid, folin reactive, methanol and $NaCO_3$ were supplied from Sigma-Aldrich and Merck.

2.2. Sample Extraction

Samples were freeze dried (Lyolab freeze dryer) and ground in a mortar. The samples (1 g) were extracted with 10 ml 80% MeOH in a shaker for one hour and filtered, while the remaining material was re-extracted with 15 ml %80 MeOH overnight. Filtered extracts were combined and concentrated in 80% MeOH with an evaporator. Dried extracts were stored at 4°C till their use. They were completed to 2 ml with %100 MeOH to determine antioxidant activity, total phenolic compounds. The methanolic, ethanolic, chloroform, acetone, and water extracts of mosses were prepared for the antimicrobial activities.

2.3. Total Phenolic Content

The total phenol amount of samples was based on Singleton et al. (1965). Folin-ciocalteu stock solution was diluted

with a 1:3 rate of water. After 20 g of sodium carbonate was dissolved in 250 ml water, it was left for one night to be filtered the next day. 250 mg gallic acid was dissolved in %10 ethanol. Solutions of this gallic acid were prepared in 15.62, 31.25, 62.5, 125, 250, 500 mg/l concentrations. From a one-gram-fresh sample, a 2 mg/ml solution was obtained. From each Sample/Gallic acid solution, 20 µl were taken. 100 µl Folin-ciocalteu solution was added in 1.58 ml of distilled water, and it stayed there for about five minutes. 300 µl %20 sodium carbonate was added in each, and they stayed in a dark place for 2 hours. Their absorbances were measured at 765 nm. Finally, the calibration curve of gallic acid absorptions was formed (Singleton and Rossi 1965). A strong correlation (R^2=0.99407) for the total phenolic content was found.

The total phenolic content of the plant extracts and the standard antioxidant materials were determined according to the Folin Ciocalteu method (Singleton et al. 1999).

2.4. Antioxidant Assessment Parameters

DPPH Free Radical Scavenging Assay

The antioxidant activity of samples was measured with the DPPH assay. The free radical scavenging effects of the extracts on the 2.2-diphenyl-1-picrylhydrazyl (DPPH) were determined according to the Sanchez-Moreno method (Wang et al. 1996). The changes in the absorbance of the specimens were measured at 517 nm with a UV spectrophotometer (Tetra), where pure methanol was used. Radical scavenging activity was expressed as the percent inhibition (%) of DPPH radical and was calculated with the following formula (Ellnain et al. 2003, Abdille et al. 2005, Mainasara et al. 2021).

The antioxidant activity (Inhibition (%) was calculated as follows:

$$\text{AAInhibition}(\%) = \frac{(\text{absorbance sample} - \text{absorbance empty sample}) \times 100}{\text{absorbance control}}$$

2.5. Antimicrobial Screening

The screening of the antimicrobial activity of the crude extract of the colloid and rhizoid of mosses were carried out individually on active cultures of *Staphylococcus aureus* ATCC 29213, *Methicillin resistant* S*taphylococcus aureus* (MRSA) ATCC 43300, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa ATCC 27853* and *Enterococcus fecalis ATCC* 29212. All strains were procured from the Ankara University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Turkey.

The minimum inhibitory concentrations (MIC) of extracts were determined with broth microdilution method according to the recommendation of European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards (ISO 20776-1 2006). For this purpose, 100 µL of Cation adjusted Mueller Hinton Broth (CMHB) (Becton Dickinson) was added to each well of 96-well microplates, then 100 µL of sample was placed in the first well to obtain double-fold dilutions. In the last stage, the bacterial suspensions, prepared equal to McFarland 0.5 turbidity with a densitometer (Biosan), was diluted 1/100 and 100 µL was added to all wells. Microplates were incubated at 37°C for 16-20 h. After incubation, the lowest concentration that inhibit the visible growth of the microorganism defined as MIC. Ciprofloxacin was used as control.

3. Result

3.1. Total Phenol Content (TPC)

The TPC of the moss extracts were expressed as mg GAE/100 g dw of extract. The methanol extracts of samples were found to have significantly higher TPC ($p \leq 0.05$). The methanol extract of *P. euchloron* showed the highest TPC (0.027±0.002 mg/g GAE/100 g) among all moss extracts. The maximum TPC in methanol extracts was determined in *P. striatum* (0.022 ± 0.002 mg/g GAE/100 g), *C. chrysophyllus* (0.008 ± 0.001 mg/g GAE/100 g) and *C. filicinum* (0.0055 ± 0.0015 mg/g GAE/100 g) (Table 2).

3.2. Antimicrobial and Antioxidant activity

The tested extracts have no antibacterial activity against the investigated microorganisms with the microdilution method (>500 µg/mL) (Table 1), however the methanol extract of mosses was found to have significant antioxidant activity and phenolic contents.

Four mosses species were screened for their potential as antioxidants using the DPPH radical scavenging activity method. The DPPH radical is widely used in assessing free radical scavenging activity because of the ease of the reaction. The % inhibition values of DPPH due to the absorbance measured at 517 nm in the radical scavenging experiments are shown in Table 1. In parallel to the examination of the antioxidant activity of plant extracts, the values for three standard compounds were obtained and then compared to the values of the antioxidant activity. The standard substances were BHA, BHT and ascorbic acid.

The free radical scavenging capacity of the four moss species in different extracts, the three positive controls viz. BHA,

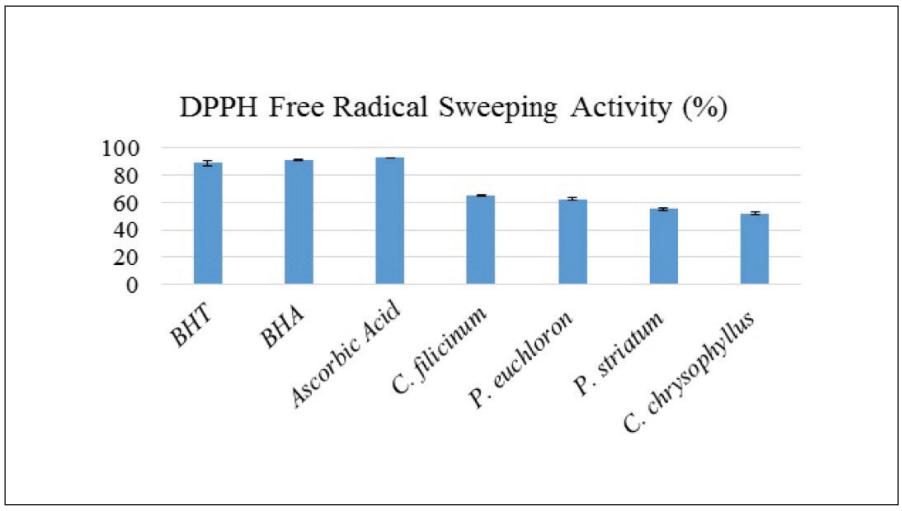

Figure 1. DPPH Free Radical Sweeping Activity (%).

Table 1. Result of the antimicrobial activity of the moss extracts with microdilution (MIC (µg/mL)).

Moss Extracts / Microbial Strain	Plasteurhynchium striatum	Palamocladium euchloron	Cratoneuron filicinum	Campyliadelphus chrysophylus	Ciprofloxacin
Staphylococcus aureus ATCC 29213	>500	>500	>500	>500	0.5
(MRSA) ATCC 43300	>500	>500	>500	>500	
Escherichia coli ATCC 25922	>500	>500	>500	>500	0.0039
Pseudomonas aeruginosa ATCC 27853	>500	>500	>500	>500	0.25
Enterococcus fecalis ATCC 29212.	>500	>500	>500	>500	

BHT, and ascorbic acid were compared through their ability to scavenge DPPH radical. The DPPH radical scavenging capacity of the bryophytes extracts and the positive controls increased in a dose dependent manner at a concentration of 0.1–0.5 mg/ml. The rate of DPPH radical scavenging capacity was found to depend on bryophytes species and extracting solvent (Kumar et al. 2013). DPPH radical scavenging capacity of methanol was significantly higher ($p<0.05$) in comparison with the other extract for most of the moss species. The maximum DPPH radical scavenging capacity in methanol extracts was retained by *C. filicinum* (%65) and *P. euchloron* (%62). Ascorbic acid (%92), BHA (%91), and BHT (%89) were found to produce a significantly higher ($p<0.05$) DPPH radical scavenging effect when compared to the moss extracts (Table 2), (Figure 1).

Table 2. Percentage inhibition of DPPH free radical of plants extract at 517nm.

Solution	DPPH Free Radical Scavenging Activity (%)
BHT	88.9227
BHA	91.2591
Ascorbic Acid	92.8188
C. filicinum	65.1127
P. euchloron	62.7124
P. striatum	55.2202
C. chrysophyllus	51.9416

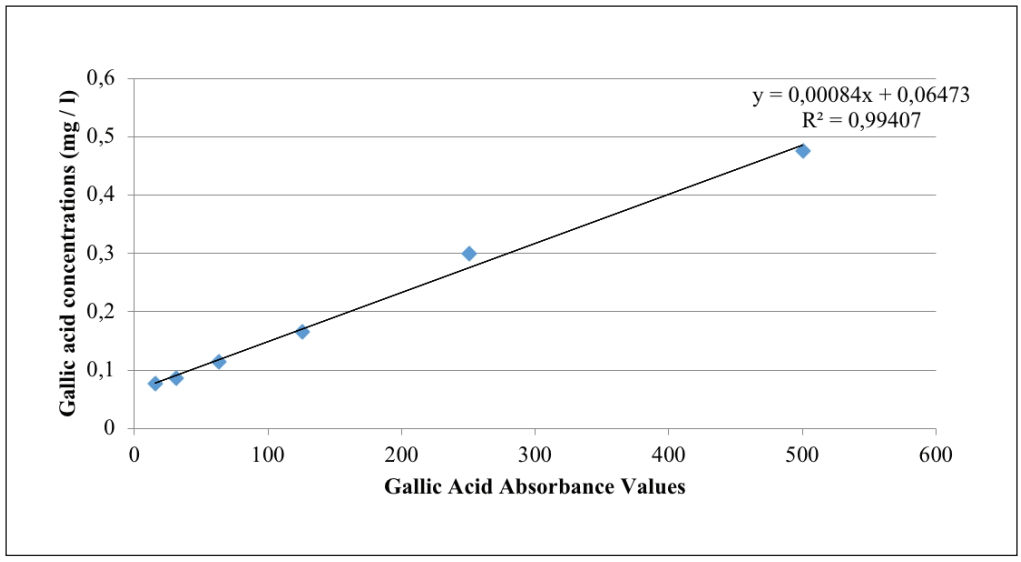

Figure 2. Prepared using the gallic acid standard for total phenolic substance identification standard working chart.

Table 3. Total phenol contents of plant extracts determined by total phenol contents.

Sample	mg/g (Gallic Acid Equivalent)
P. euchloron Average	0.027±0.002 mg/g
P. striatum Average	0.022 ± 0.002 mg/g
C. chrysophyllus Average	0.008 ± 0.001 mg/g
C. filicinum Average	0.0055 ± 0.0015 mg/g

The total phenolic contents in the plant extracts were expressed in terms of gallic acid equivalent using the Folin-Ciocalteu's reagent. The values obtained for the concentration of total phenols were expressed as mg of GA/g of extract (Table 3), (Figure 2).

4. Discussion

Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari and Chatterjee 2008). In this study, the percentages of inhibition of the antioxidative activity of four moss species were determined and also contributed significantly to literature due to lack of studies evaluating the antioxidative properties of moss extracts.

The most important groups of natural antioxidants are phenolic substances (Tunalıer et al. 2002). Therefore, the determination of the quantity of phenolic compounds is very crucial to understand the antioxidant capacity of plant extracts (Das and Pereira 1990, De Gaulejac et al. 1999, Sabovljević et al. 2001). Total phenolic content was determined by using the Folin-Ciocalteu reagent. The total phenolic content of the different fractions of plants was solvent dependent and expressed as milligrams of gallic acid equivalents (GAE) in all the plant extracts. In studies it was indicated that the total phenolic concentration in plant material was directly associated with antioxidant activity. (Velioglu et al. 1998). The total phenolic compounds in fractions varied widely, ranging from 0.008 ± 0.001 mg/g to 0.027±0.002 mg/g were expressed as gallic acid equivalents (GAE). *P. euchloron* exhibited the highest total phenolic content.

Also, the antioxidant activities of the extracts were compared with BHT and BHA, which are synthetic antioxidants. Furthermore, the antioxidant activities of the extracts were compared with ascorbic acid that is a natural antioxidant. Ascorbic acid (%92), BHA (%91), and BHT (%89) were found to produce a significantly higher ($p<0.05$) DPPH radical scavenging effect when compared to the bryophyte extracts. The maximum DPPH radical scavenging capacity in methanol extracts was retained by *C. filicinum* (%65) and *P. euchloron* (%62). Highly radical scavenging effects of different concentrations of *C. filicinum* was demonstrated in this study. However, the radical scavenging effects of *P. striatum*, *P. euchloron* and *C. chrysophylus* were not found as great as that of *C. filicinum*.

Only %5 of the bryophytes have been chemically analyzed in the literature so far in the World (Sabovljevic et al. 2012). These studies are increasing day by day. It is considered that

bryophytes will become more important in the future due to have a wide variety of phenolic compounds and antioxidant effects, and to be a potential sources in the future.

Similarly, in our literature survey we found no study related phytochemical content of the *P. striatum, P.euchloron, C. filicinum* and *C. chrysophyllus* species.

When the scientific researches carried out up to now are examined, the DPPH radical used in the DPPH method is used for the differences in the concentrations of plant extracts used in the research, DPPH radical or antioxidants may vary depending on exposure to light and air. It is clear that different results are obtained in this case (Mishra et al. 2012).

An extract derived from *Perilla frutescens* sprouts was shown to exhibit potent antioxidative properties, including the ability to effectively scavenge DPPH, anti-inflammatory activity, and antiemetic effects. So, four moss species in this study may have similar effects as *P.frutescens.* (Jeong et al. 2014).

4.1. Disclosure Statement

No potential conflict of interest was reported by the authors.

5. References

Abdille, H., Singh, RP., Jayaprakasha, GK., Jena, BS. 2005. Antioxidant activity of the extracts from *Dillenia indica* fruits. Food Chem., 90:891-896. Doi: 10.1016/j.foodchem.2004.09.002.

Bhattarai, HD., Paudel, B., Lee, HK., Joung, H., Yima, H. 2009. In vitro antioxidant capacities of two benzonaphthoxanthenones: Ohioensins F and G isolated from the antarctic moss *Polytrichastrum alpinum*. Z. Naturforsch., 64:197-200. Doi: 10.1515/znc-2009-3-408

Bodade, RG., Borkar, PS., Arfeen, MS., Khobragade, CN. 2008. In vitro screening of bryophytes for antimicrobial activity. Journal of Medicinal Plants., 7:23-28.

Cansu, T.B. Yaylı, T. Özdemir, N. Batan, Ş. Alpay-Karaoğlu ve N. Yaylı "Antimicrobial activity and chemical composition of the essential oils of mosses (*Hylocomium splendens* (Hedw.) Schimp. and *Leucodon sciuroides* (Hedw.) Schwägr.) growing in Turkey", Turkish journal of Chemistry, 37(2), 213-219, (2013).

Cansu, T.B. Özdemir, N. Batan, B. Yaylı, Ş. Alpay-Karaoğlu ve N. Yaylı "Essential Oil Analysis and Antimicrobial Activity of *Neckera complanata* (Hedw.) Huebener and *Neckera crispa* Hedw. (*Neckeraceae*) Grown in Turkey", Asian Journal of Chemistry, 26 (7), 2005-2008, (2014).

Chobota, V., Kubicova, L., Nabbout, S., Jahodar, L., Hadacek, F. 2008. Evaluation of antioxidant activity of some common mosses. Z Naturforsch C J Biosci., 63. 476-482. Doi: 10.1515/znc-2008-7-802.

Cui, Y., Kim, DS., Park, KC. 2005. Antioxidant effect of Inonotus obliquus. J. Ethnopharmacol., 96:79-85. Doi: 10.1016/j.jep.2004.08.037

Cvetić, T., Sabovljević, A., Pristov, JB., Sabovljević, M. 2009. Effects of day length on photosynthetic pigments and antioxidative metabolism of in vitro cultured moss Atrichum undulatum Hedw. P. Beauv. (Bryophyta). *Bot Serb*., 33(1):83-88.

Çöteli E, Alataş M, Batan N. 2017. "*Syntrichia ruralis* ve *Syntrichia montana* (Pottiaceae) Taksonlarının Glutatyon İçeriklerinin Karşılaştırılması", Anataolian Bryology, 3(1): 25-30.

Çöteli E, Alataş M, Batan N, Hazer Y. 2019. "Comparing of Glutathione Ingredients of Some Species in Bryaceae Family", Anatolian Bryology, 5(1): 15-21.

Das, NP., Pereira, TA. 1990. Effects of flavonoids on thermal autooxidation of palm oil: structure–activity relationship. J. Am. Oil Chem. Soc., 67:255-258.

De Gaulejac, NSC., Glories, Y., Vivas, N. 1999. Free radical scavenging effect of anthocyanins in red wines. Food Res. Int., 32:327-333. Doi: 10.1016/S0963-9969(99)00093-9.

Ellnain-Wojtaszek, M., Kruczynski, Z., Kasprzak, J. 2003. Investigation of the free radical scavenging activitiy of *Ginkgo biloba* L. leaves. Fitoterapia, 74:1-6. Doi: 10.1016/s0367-326x(02)00306-4.

Glime, JM. 2007. Economic and ethnic uses of bryophytes. In flora of North America Editorial Committee, ed. Flora of North America, North of Mexico. Bryophyta 27:14-41.

Goffinet, B., Shaw, AJ. 2009. Bryophyte biology, 2nd ed., Cambridge University Press, The Edinburgh Building, Cambridge CB2 8RU, UK, 565. Ann. Bot., 104:vi–x. Doi: 10.1093/aob/mcp109.

Hinneburg, I., Dorman, HJG., Hiltunen, R. 2006. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem., 97:122-129. Doi: 10.1016/j.foodchem.2005.03.028

Hsiao, G., Teng, CM., Wu, CL., Ko, FN. 1996. Marchantin H as a natural antioxidant and free radical scavenger. Arch. Biochem. Biophys., 334(1):18-26. Doi: 10.1006/abbi.1996.0424.

International Organization for Standardization. 2006. ISO 20776-1: Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices -Part 1: Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

Jeong, SI., Kim, HS., Jeon, IH., Kang, HJ., Mok, JY., Cheon, CJ., Yu, HH., Jang, SI. 2014. Antioxidant and anti-inflammatory effects of ethanol extracts from *Perilla frutescens*. *Korean* J Food Sci Technol., 46:87-93. Doi: 10.9721/KJFST.2014.46.1.87.

Kumar, J., Dhar, P., Tayade, AB., Gupta, D., Chaurasia, OP., Upreti, DK., Arora, R., Srivastava, RB. 2014. Antioxidant Capacities, Phenolic Profile and Cytotoxic Effects of Saxicolous Lichens from Trans-Himalayan Cold Desert of Ladakh. PloS One., 9:6. Doi: 10.1371/journal.pone.0098696.

Magtoto, LM., Alonzo, EAM., Lacerna, MZT., Santiago, JC. 2015. Total Phenol Content and Antioxidant Activity of *Bryum billardieri* Schwaegr. IJCPR., 7(1):19-20.

Mainasara, MM., Abu Bakar, MF., Md Akim, A., Linatoc, AC., Abu Bakar, FI., Ranneh, YKH. 2021. Secondary Metabolites, Antioxidant, and Antiproliferative Activities of *Dioscorea bulbifera* Leaf Collected from Endau Rompin, Johor, Malaysia. Based Complement Alternat Med., 2021:8826986. Doi: 10.1155/2021/8826986.

Mishra, K., Ojha, H., Chaudhury, NK. 2012. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chem., 130:1036-1043. Doi: 10.1016/j.foodchem.2011.07.127.

Pejin, B., Bogdanović-Pristov, J. 2012. ABTS cation scavenging activity and total phenolic content of three moss species. Hem Ind., 66(5):723-726. Doi: 10.2298/hemind120131022p.

Pejin, B., Bogdanovic-Pristov, J., Pejin, I., Sabovljevic, M. 2013. Potential antioxidant activity of the moss *Bryum moravicum*. Nat. Prod. Res., 27(10):900-902. Doi: 10.1080/14786419.2012.665915.

Pejin, B., Savic, A., Kien-Thai, Y., Mojovic, M. 2014. Further *in vitro* evaluation of antiradical activity of the moss *Rhodobryum ontariense* tea using EPR and fluorescence spectroscopy. Cryptogamie Bryol., 35(2):173-179. Doi: 10.7872/cryb.v35.iss2.2014.173.

Rice-Evans, CA., Miller, NJ., Bolwell, PG., Bramley, PM., Pridham, JB. 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res., 22:375-383. Doi: 10.3109/10715769509145649.

Sabovljević, M., Bijelović, A., Grubišić, D. 2001. Bryophytes as a potential source of medicinal compounds. Lek. Sirov., 21:17-29.

Sabovljević, A., Vujicic, M., Skoric, M., Bajic-Ljubicic, J., Sabovljević, M. 2012. Axenically culturing the bryophytes: Establishment and propagation of the pleurocarpous moss *Thamnobryum alopecurum* Nieuwland ex Gangulee (Bryophyta, Neckeraceae) in *in vitro* conditions. Pak. J. Bot., 44(1):339-344.

Shai, LJ., Masoko, P., Mokgotho, MP., Magano, SR., Mogale, AM., Boaduo, N., Eloff, JN. 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa. S. Afr. J. Bot., 76:465-470. Doi: 10.1016/j.sajb.2010.03.002.

Singleton, VL., Rossi, JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16:144-158.

Singleton, V., Orthofer, R., Lamuela-Ravento´s, R. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In L. Packer (Ed.). Oxidants and antioxidants, part A. Meth. Enzymol., 299: pp. 152–178. New York: Academic Press. Doi: 10.1016/S0076-6879(99)99017-1.

Tosun, G.B. Yaylı, T. Özdemir, N. Batan, N. Yaylı ve Ş. Alpay-Karaoğlu "Chemical Composition and Antimicrobial Activity of the Essential Oils from *Tortella inclinata* var. *densa*, *T. tortusa* and *Pleurochaete squarrosa*", Asian Journal of Chemistry, 26(7), 2001-2004, (2014).

Tosun, G.B. Yayli, T. Özdemir ve N. Batan, A. Bozdeveci ve N. Yayli "Volatiles And Antimicrobial Activity of the Essential Oils of the Mosses *Pseudoscleropodium purum*, *Eurhynchium striatum*, and *Eurhynchium angustirete* Grown in Turkey", Records of Natural Products, vol.9, pp.237-242, (2015).

Tunalıer, Z., Öztürk, N., Koşar, M., Başer, KHC., Duman, H., Kırımer, N. 2002: Bazı Sideritis türlerinin antioksidan etki ve fenolik bileşikler yönünden incelenmesi. Bitkisel İlaç Hammaddeleri Toplantısı, s. 76-93, Eskişehir.

Umamaheswari, M., Chatterjee, TK. 2008. *In vitro* antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. Afr. J. Trad. Compl. Altern. Med., 5:61-73.

Velioglu, YS., Mazza, G., Gao, L., Oomah, BD. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agri. Food Chem. 46:4113-4117. Doi: 10.1021/jf9801973.

Yağlıoğlu, MS., Abay, G., Demirtaş, I., Yağlıoğlu, AS. 2017. Phytochemical screening, antiproliferative and cytotoxic activities of the mosses *Rhytidiadelphus triquetrus* (Hedw.) Warnst. and *Tortella tortuosa* (Hedw.) Limpr. Anatolian Bryol. 3(1):31-42. Doi: 10.26672/anatolianbryology.317785

Wang, H., Cao, G., Prior, RL. 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem., 44:701-705. Doi: 10.1021/jf950579y.