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Determination of Phytochemical Content and Antioxidant Activities of *Sphagnum divinum* Flatberg & K. Hassel and *Sphagnum girgensohnii* Russow (Sphagnopsida)

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Abstract

This study aimed to explore the phytochemical constituents and antioxidant activity of methanol extracts from Sphagnum divinum and Sphagnum girgensohnii. Screening methods were employed to identify the phytochemical groups present in the methanol extracts of these two Sphagnum species, which have been relatively understudied for their antioxidant potential. The antioxidant properties of the extracts were evaluated using in vitro DPPH, CUPRAC, and phosphomolybdate assays. The experimental results revealed that the methanol extract of S. divinum exclusively contained phenols and tannins, while the methanol extract of S. girgensohnii contained phenols, tannins, and saponins. Alkaloids and flavonoids were not detected in either bryophyte. The methanol extracts of both S. divinum and S. girgensohnii exhibited DPPH scavenging activity exceeding 50% at all tested concentrations. An increase in DPPH scavenging ability was observed with increasing extract concentration. The DPPH scavenging activity of S. divinum methanol extract was consistently higher than that of S. girgensohnii methanol extract across all tested concentrations. These findings suggest that S. divinum and S. girgensohnii hold promise as potential sources of antioxidant compounds.

Key words: Bryophytes, Peatland, CUPRAC assay, DPPH, Phosphomolybdate assay

Sphagnum divinum Flatberg & K. Hassel ve *Sphagnum girgensohnii* Russow (Sphagnopsida)'nin Fitokimyasal İçeriklerinin ve Antioksidan Aktivitelerinin Belirlenmesi

Öz

Bu çalışmanın amacı, *Sphagnum divinum* ve *S. girgensohnii*'den elde edilen metanol ekstraktlarının fitokimyasal bileşenlerini ve antioksidan aktivitesini araştırmaktır. Antioksidan içeriği çok az çalışılmış olan iki farklı Sphagnum türünün metanol ekstraktındaki fitokimyasal aktif madde grupları tarama yöntemleri kullanılarak belirlenmiş ve antioksidan özellikleri *in vitro* DPPH deneyi, CUPRAC deneyi ve fosfomolibdat deneyi kullanılarak tespit edilmiştir. Deneylerden elde edilen sonuçlara göre, *S. divinum* 'un metanol ekstraktının sadece fenoller ve tananler içerdiği, *S. girgensohnii* 'nin metanol ekstraktının ise fenoller, tananler ve saponinler içerdiği, ancak her iki briyofitin de alkaloidler ve flavonoidler içermediği belirlenmiştir. Deneysel sonuçlar, *S. divinum* ve *S.girgensohnii* metanol ekstraktlarının test edilen tüm konsantrasyonlarda %50'den daha yüksek DPPH süpürme aktivitesine sahip olduğunu göstermektedir. Ekstrakt konsantrasyonundaki artışla birlikte DPPH süpürme kabiliyetinde artış olduğu gözlemlenmiştir. *S. divinum* metanol ekstraktının DPPH süpürme kabiliyeti, test edilen tüm konsantrasyonlarda *S. girgensohnii* metanol ekstraktından daha yüksek bulunmuştur. Çalışmanın sonuçları, *S.divinum ve S. girgensohnii*'nin potansiyel antioksidan bileşik kaynakları olabileceğini göstermektedir.

Anahtar kelimeler: Karayosunları, Turbalık, CUPRAC Testi, DPPH, Fosfomolibdat testi

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1. Introductıon

It is known that the antioxidant properties of plants are effective in defense against oxidative destruction in cells, in different diseases such as cancer, vascular occlusion, and in protection against the aging process. Especially the development and utilization of natural antioxidant species are among the important research topics today. Although medicinal plants used in traditional medicine are preferred as natural antioxidant sources, it is a fact that new and different sources with high natural antioxidant capacity are needed today.

Approximately 1030 taxa in Türkiye represented bryophytes, which make up the second largest group of our plant biodiversity, according to 2015 data. According to the last updated bryophyte lists (Erdağ and Kürschner 2017; Kürschner and Frey 2020) and recent studies on bryophytes (Alataş et al., 2019; Batan et al., 2019; Ellis et al., 2019, 2020, 2021; Ursavaş and Işin 2019; Ursavaş and Keçeli 2019; Erata and Batan 2020; Erata et al., 2020; Uygur et al., 2020; Unan et al., 2020; Abay et al., 2020; Erata et al., 2021; Unan and Ören 2021; Ursavaş et al., 2021; Kırmacı et al., 2021, 2022; Özenoğlu and Kırmacı, 2022; Özen-Öztürk et al., 2023) including the studies conducted in the period from 1829 until the end of 2020, existing flora has reached ±1047 taxa (±844 mosses, ±199 liverworts and 4 hornworts).

It is known that bryophytes produce various secondary metabolites to combat many stresses such as insect/animal predation, UV radiation, extreme temperature, and microbial decomposition. Secondary metabolites are compounds that do not directly affect the basic activities of plants but have primary metabolites directly related to basic activities (Shaw and Goffinet, 2000). Secondary metabolites have anti-cancer, anti-tumor, anti-fungal, anti-bacterial, and anti-oxidant activities and the anti-oxidant capacity of bryophytes is higher than some higher-structured plants (Xie and Lou, 2009). Because of reasons such as the region of distribution, seasonal changes, the amount of water and humidity to which it is exposed, and the substances taken from the environment, the secondary compounds in their content may vary according to the species and may show a rich diversity (Heinrichs et al., 2000).

Secondary metabolites are produced biosynthetically from primary metabolites in plants. Among the secondary compounds found in the structure of bryophytes are aromatic compounds, terpenoids, and fatty acids. Besides terpenoids, aromatic compounds are the most important type of secondary metabolite found in bryophytes. The most commonly observed flavonoid species in bryophytes are flavone aglycones and glycosides, flavonol aglycones and glycosides, anthocyanins and derivatives, aurones, biflavonoids, flavanones, dihydrochalcones, dihydroflavonols, isoflavones, triflavones (Cowan, 1999., Dey and De, 2012). Also aromatic compounds, terpenoids and fatty acids (Serin, 2007), aromatic compounds, benzoic and cinnamic acid derivatives, phenolethers, alkylphenols, phenylglycosides, bisbibenzyls, bisbibenzyldimers, stilbenes, phenanthrenes, naphthalenes, acetophenones, lignans, coumarins, isocoumarins, comestanes, and benzonaphthoxanthenones (Heinrichs et al, 2000). These secondary compounds in bryophytes stand out with their anti-cancer, anti-tumor, anti-fungal, anti-bacterial and anti-oxidant properties (Bhattarai et al., 2009; Gaurav et al., 2018; Provenzano et al., 2019; Başer Canoğlu et al., 2019). There are many bryophytes with antioxidant potential and it is known that the antioxidant capacity of bryophytes is higher than some highly structured plants (Mukhopadhyay et al., 2013; Vats and Alam, 2013; Cansu et al., 2013; Çelik et al., 2014; Oyedapo et al., 2015; Aslanbaba et al., 2017; Karaoğlu et al., 2022; Karaoğlu et al., 2023; Çelik et al., 2023). Studies conducted with different species of bryophytes have revealed that bryophytes can be used as sources of antioxidants which are very useful in medical terms. Although there are examples of studies showing that flavonoids and phenolic compounds in the composition of black algae can affect the antioxidant activity of bryophytes, studies on this subject are very new and not yet sufficient.

Sphagnum is commonly referred to as peat moss because of its ecological significance in creating peat and bog. There are very limited bogs created by sphagnums in Türkiye and all of these are located in the north-eastern part of Türkiye (North-Eastern Black Sea Region) (Kırmacı et al., 2019). It represented the genus *Sphagnum* with 26 taxa in Turkish bryophyte flora (Kırmacı et al., 2022). In this study, two *Sphagnum* species, *S. divinum* and *S. girgensohnii*, were studied from Türkiye. *S. girgensohnii* is more common than *S. divinum* which has recently been given as a new record for the country (Ellis et al., 2019).

Studies on bryophytes in our country are mostly for bryofloristic purposes and studies on their biological characteristics are very limited. In this study, methanol extracts of two species belonging to two different taxa of the genus *Sphagnum*, of which there are very few studies on the antioxidant content, were got and the phytochemical active

substance groups in these crude extracts were investigated using screening methods. The antioxidant properties of the extracts were determined using *in vitro* antioxidant methods such as free radical scavenging (DPPH assay), reducing power (CUPRAC assay), and phosphomolybdate assay.

2. Materials and methods

2.1. Chemicals

1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), Ascorbic acid, and methanol were purchased from Sigma, Chem., Germany and CUPRAC assay kit was purchased from BQC (Bioquochem) Redox Technologies, Asturias, Spain.

2.2. Collection Localities

S. girgensohnii: Trabzon, Sürmene, Ağaçbaşı Yaylası, 2000 m., N 40° 42′ 24.2″ E 0.40° 05′ 40.6″,, Collection date: 08.09.2021, MKIR 8557
S. divinum: Rize, Kavrun Yaylası, 2050m; N 40°θ 53′ 49.8″ E 41°θ 07′ 48.4″, Collection date: 09.09.2021, MKIR 8563

2.3. Preparation of methanol extracts from *S. divinum* and *S. girgensohnii*

15g powdered *S. divinum* and *S. girgensohnii* mosses were extracted with 500 mL methanol at room temperature for 24-48h. After filtration, the extracts were evaporated and yielded 0.3863 g and 0.8581g dried mass, respectively. The crude extracts were kept at +4°C until the experimental studies.

2.4 Preliminary phytochemical screening

Phytochemical analyses were carried out according to Ravishankara (2002) and Dominguez (1973). The details of the tests as are follows:

2.4.1 Detection of phenols

Methanol extracts prepared in ethanol were spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spots and was exposed to ammonia vapors. Blue coloration of the spots indicates the presence of phenols.

2.4.2 Detection of tannins

To 2-3mL of methanolic extract, 10%alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicates the presence of tannins in the extracts.

2.4.3 Detection of alkaloids

A drop of extracts prepared i methanol was spotted in a small piece of precoate TLC plate and the plate was sprayed with Dragendorff's reagent. Orange coloration of the spot indicates the presence of alkaloids.

2.4.5 Detection of flavonoids

To 2-3 ml of the extracts prepared in methanol, a piece of magnesium ribbon and 1mL of concentrated hydrochloric acid were added. Pink-red or red coloration of the solution indicates the presence of flavonoids.

2.4.6 Detection of saponins

10mg of the extracts were mixed with hot water and the mixtures were shaken for 30s. The formation of stable foam indicates the presence of saponins.

2.5. Folin-Ciocalteu assay for total phenolic content

Samples were analyzed spectrophotometrically for contents of total phenolic by a *Folin– Ciocalteu* colorimetric method (Singleton et al., 1999). 100 and 300µL of properly diluted extract solutions were mixed with 1mL of FC reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 3min at room temperature, 3mL of (2 %w/v) sodium carbonate solution was added. The solutions were mixed and allowed to stand for 2h at room temperature. Then, the absorbance was measured at 760nm, using a UV spectrophotometer (Shimadzu PharmaSpec UV1700, Japan). A calibration curve was prepared, using a standard solution of gallic acid (20, 40, 60, 80 and 100mgmL^{-1}). The results were expressed as mg gallic acid per 100gram dry mosses.

2.6. Determination of Antioxidant Activity

2.6.1. DPPH free radical scavenging assay

The free radical scavenging activity of *S. divinum* and *S. girgensohnii* methanol extracts were tested for their ability to bleach the stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) (Brand-Williams et al.,1995). This test is based on the change in purple color of DPPH solution to yellow by antioxidant molecules, because of the scavenging of stable free DPPH radicals, which from purple to yellow measured at 517 nm (Blois, 1958). A stronger yellow color shows a greater ability of the extract to scavenge free DPPH radicals and stronger antioxidant potential. One milliliter of 0.1 mM DPPH methanol solution was added to 3 ml of 5, 10, 20, 40, 60, 80, and 100µgmL^{-1}concentrations of methanol extracts. The mixtures were vigorously shaken, then left at room temperature to stand. Using a micro-plate reader (Elisa Reader, Biotek Co, USA) the absorbance of the mixture was measured at λ = 517nm after 30min. 10 and 50µgmL^{-1} ascorbic acid, the commercially known antioxidant was used as a positive control. We performed all experiments in triplicate. We

calculated the percentage of the DPPH free radical using the following equation:

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the methanol extracts. We compared the actual decrease in absorption induced by the test with the positive controls.
DPPH Scavenging capacity (%) = [(A0 − A1)/A0] × 100

2.6.2. Phosphomolybdate assay (total antioxidant capacity)
The total antioxidant capacity (TAC) assay of samples was carried out by the phosphomolybdenum method (Ghafoor and Choi, 2009). A 0.1mL aliquot of the extract (10, 20, 40, 60, 80, and 100µgmL^{-1}) solution was shaken with 1mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The test tubes were covered with aluminum foil and incubated in a water bath at 95 °C for 90 min. After the samples were cooled, the absorbances were measured at 765nm. 10 and 50µgmL^{-1} ascorbic acid was used as standard. All experiments were performed in triplicate. The total antioxidant capacity (TAC) of the extracts was estimated using the following formula:
Total antioxidant capacity (%) = [(Abs. of control−Abs. of sample)/ (Abs. of control)] ×100

2.6.3. CUPRAC spectrophotometric assay of total antioxidant capacity
The total antioxidant capacity of extracts was also measured using the BQC CUPRAC assay kit (Asturias, Spain), based on the oxidation of the copper (II)-neocuproine (2,9-dimethyl-1,10-phenanthroline) according to the manufacturer's protocol. A total of 40µL of the diluted extracts was added to 200µL of the previously prepared working solution. Trolox was used as standard in experiments. The mixture was incubated at room temperature for 30min, and the absorbance was measured at 450nm using a plate reader (Elisa Reader, Biotek Co, USA). Results were expressed as mM of Trolox equivalents (TE mM).

2.7. Statistical Analysis
Each experiment was performed in three replicates. Results were expressed as means ±SD and analyzed using One-way ANOVA (Analysis of Variance) in the SPSS 25.0 (USA) software package program. Level of significance was set at $p \leq 0.05$.

3. Results

3.1 Phytochemicals in extracts
The qualitative evaluation revealed the presence of all phytochemical classes analyzed in the methanol extract. However, only phenols and tannins were found in the methanol extract of *S. divinum* and only phenols, tannins, and saponins were found in the methanol extract of *S. girgensohnii*, while alkaloids and flavonoids were absent in the methanol extract of both mosses (Table 1).

Table 1. Preliminary phytochemical screening of methanol extracts of *S. divinum* and *S. girgensohnii*

Taxa	Phenols	Tannins	Alkaloids	Flavonoids	Saponins
S. divinum	+	+	-	-	-
S. girgensohnii	+	+	-	-	+

(+): present, (-): absent

3.2 Total phenolic content
The total phenolic contents of *S. divinum* and *S. girgensohnii* methanol extracts were presented as mg of GA/g in Table 2.

Table 2. Total phenolic content of *S. divinum* and *S. girgensohnii* methanol extracts

Taxa	mg phenolic/g extract ± SD
S. divinum	50.00 ± 4.33
S.girgensohnii	52.50 ± 2.50

Each value is the average of three analyses ± standard deviation

The total phenolic contents in extracts were 50.00±4.336 mg GAE/g in *S. divinum* methanol extract, and 52.50±2.50 mg GAE/g in *S. girgensohnii* methanol extract. Phenol concentration in *S. girgensohnii* methanol extract was found higher than in *S. divinum* methanol extract. However, the difference is not statistically significant.

3.3. Antioxidant activity
3.3.1. DPPH scavenging activity
The antioxidant potential of *S. divinum* and *S. girgensohnii* methanol extracts was evaluated on the basis of their ability to scavenge stable free DPPH radicals. In the present study, the results of the antioxidant potential of methanol extracts of *S. divinum* and *S. gingensohni* obtained at different concentrations (10-100 µgmL^{-1}) are given in Table 3. Experiment results show that all concentrations of *S. divinum* and *S. girgensohnii* methanol extracts have DPPH scavenging activity higher

than 50%. We observed an increase in DPPH scavenging ability with an increase in the concentration of extracts. The DPPH scavenging ability of *S. divinum* methanol extract was higher than that of *S. girgensohnii* methanol extract at each concentration tested. 10µgmL^{-1} concentration and 50µgmL^{-1} concentrations of ascorbic acid used as standard in the experiment also showed 81.56% and 82.79% DPPH scavenging activity, respectively.

Table 3. Antioxidant activities of *S. divinum* and *S. girgensohnii* methanol extracts

Groups	**Concentrations**	**DPPH scavenging activity (%±SD)**	**Total Antioxidant capacity (%±SD)**
Askorbik asit	10 µgmL^{-1}	81.56±0.001*	36.16±0.013
	50 µgmL^{-1}	82.79±0.001*	97.98±0.008*
S. divinum	10 µgmL^{-1}	65.16±0.003*	45.19±0.013
	20 µgmL^{-1}	69.67±0.002*	59.75±0.017*
	40 µgmL^{-1}	72.13±0.002*	61.58±0.035*
	60 µgmL^{-1}	72.95±0.003*	65.93±0.045*
	80 µgmL^{-1}	77.05±0.002*	70.06±0.034*
	100 µgmL^{-1}	77.46±0.008*	78.79±0.014*
S. girgensohnii	10 µgmL^{-1}	61.07±0.004*	41.81±0.021
	20 µgmL^{-1}	64.75±0.002*	55.93±0.020*
	40 µgmL^{-1}	65.98±0.002*	83.40±0.025*
	60 µgmL^{-1}	67.62±0.002*	84.65±0.007*
	80 µgmL^{-1}	70.49±0.001*	85.48±0.039*
	100 µgmL^{-1}	70.49±0.019*	87.57±0.020*

*p<0.05

3.3.2. Total antioxidant capacity (TAC)

The total antioxidant capacity results determined by the phosphomolybdate assay are given in Table 3. According to the results, both methanol extracts showed high total antioxidant capacity from 20µg mL^{-1} concentration, which increased depending on the concentration increase. The total antioxidant capacity of *S. girgensohnii* methanol extract was found to be higher than that of *S. divinum* methanol extract, and the difference was statistically significant (p<0.05). While 10µg mL^{-1} concentration of ascorbic acid used as a standard in the experiment showed 36.16 % total antioxidant capacity, this value showed a significant increase and reached 97.98% at 20µg mL^{-1} concentration.

3.3.3. CUPRIC Ion Reducing Antioxidant Capacity

The total antioxidant capacity of *S. divinum* and *S. girgensohnii* methanol extracts was also measured using the BQC CUPRAC assay kit (Asturias, Spain) based on the oxidation of copper (II)-neocuproine (2,9-dimethyl-1,10-phenanthroline) according to the manufacturer's protocol. The results of the CUPRAC method showed that the methanol extracts of *S. divinum* and *S. girgensohnii* had very low antioxidant activity compared to Trolox, which was used as a standard in the experiment (Table 4, Fig. 1-3).

Tablo 4. Total antioxidant capacity of *S. divinum* and *S. girgensohnii* methanol extracts measured by CUPRAC assay

Taxa	**CUPRAC value (mM TR/g)**
S. divinum	0.0008
S. girgensohnii	0.0003
Troloks (Standart)	1,000

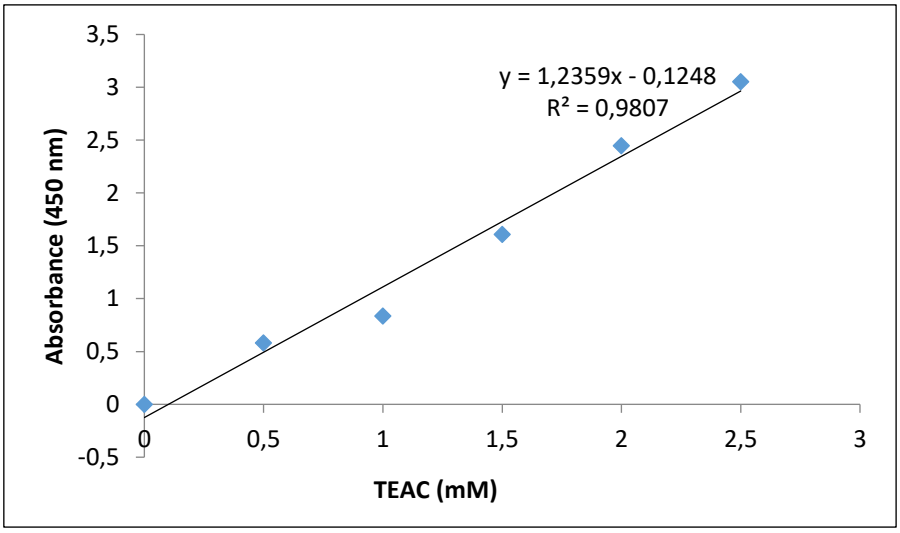

Figure 1. CUPRAC standard run graph for Trolox

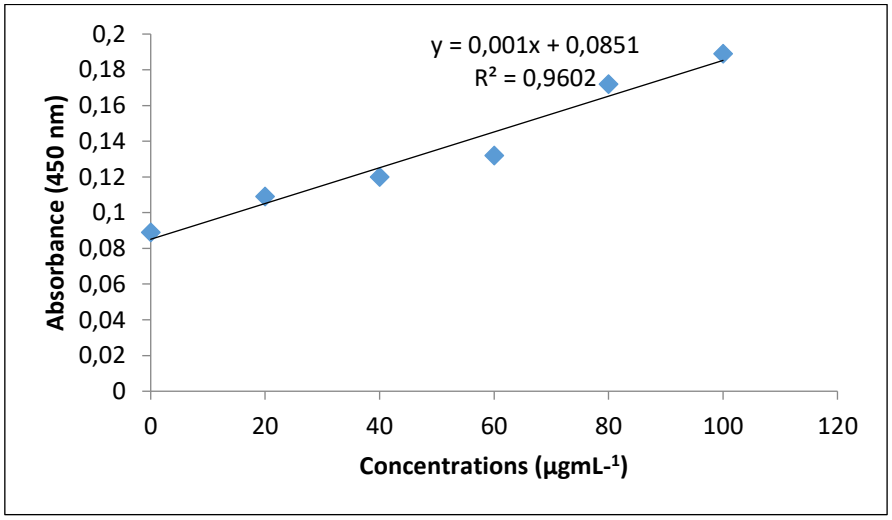

Figure 2. CUPRAC activity for *S. divinum* methanol extract

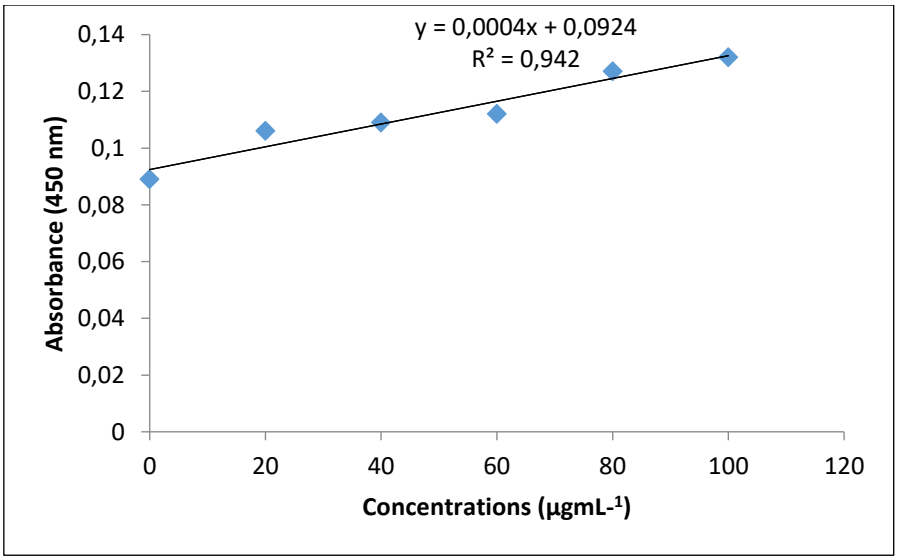

Figure 3. CUPRAC activity for *S. girgensohnii* methanol extract

4. Discussion

It is known that oxidized free radicals cause various degenerative diseases. Therefore, there is a great need to search for effective natural antioxidants to combat the onset of degenerative diseases and aging. The phytochemical from got from various parts of plants are the major source of antioxidants. These phytochemicals play a role in the defense mechanisms of plants and in maintaining the redox balance in the body and protection from various diseases (Kandpal et al., 2016). In recent years, biologically active ingredients of plant origin have become crucial as highly promising, prophylactic, and restorative measures to combat diseases caused by oxidative stress. Although many studies have been carried out on flowering plants related to the subject, there are limited studies on cryptogams. Bryophytes, which consist of the largest group of cryptogams have not yet been much investigated, although they have a non-woody structure and possess unique phytochemicals that provide a strong defense mechanism for survival under a wide variety of habitats.

Bryophytes are rich in phenols (flavonoids and ibibenzyl derivatives), terpenoids, glucose, fatty acids and some aromatic compounds. Moreover, many of them have biological activity and contain novel natural products or secondary compounds that may provide great potential for bio-pharmaceutical applications (Krishnaiah et al., 2007; Vats and Alam, 2013; Cansu et al., 2013; Çelik et al., 2014; Aslanbaba et al., 2017; Gahtori and Chaturvedi, 2020; Karaoğlu et al., 2022; Karaoğlu et al., 2023; Çelik et al., 2023). Especially in Indian and Chinese medicine, liverwort, which is included in bryophytes, is an important natural product storehouse for the treatment of hepatitis and skin diseases (Gökbulut et al, 2012). Although mosses are more diverse than liverworts, they have been relatively less investigated in terms of medicinal usefulness.

Studies have shown that bryophytes are good sources of phytochemicals, nutrients, mineral elements, and cosmetics. Bryophytes produce several of the secondary metabolites that empower these delicate plants with powerful anti-oxidative mechanisms to cope with biotic and abiotic stresses (Xie and Lou, 2009; Dey and De, 2012). Secondary metabolites protect plants against oxidative stress, compensating for the absence of any morphological and anatomical defence mechanisms. Under unfavourable conditions, reactive oxygen species react with important cellular components such as proteins and lipids, leading to disruption of cell structure and cell damage. Antioxidant enzymes protect the cell membrane and cell organelles against oxidative stress caused by both internal and external adverse conditions. It is thought that the high amount of antioxidants found in liverworts and mosses can be used as a future source of medicinal and cosmetic properties (Aslanbaba et al., 2017). However, research results on the biological properties of mosses belonging to *Sphagnum* species are limited. Therefore, in this study, qualitative phytochemical screening, total phenolic content (GAE/gm), antioxidant activity, and total antioxidant capacity of methanol extracts from *S. divinum* and *S. girgensohnii* were determined by different methods.

Biologically active components found in crude extracts of plants are known directly responsible for different activities, such as anti-oxidant, anti-microbial, anti-fungal, and anti-cancer. (Harborne, 1998., Beer et al., 2007., Hossain and Nagooru, 2011). All these secondary metabolites showed antioxidant and antimicrobial properties through different mechanisms (Hossain et al., 2011).

Previous investigations have shown that bryophytes possess an exorbitant amount of secondary metabolites such as terpenoids, phenolics (flavonoids and bi-benzyl derivatives), glycosides, fatty acids and some rare aromatic compounds (Sabovljvic et al., 2019). In some studies, it was reported that flavonoids were found in methanol, ethanol, and petroleum ether extracts got from bryophytes, but not in chloroform and acetone extracts. Qualitative phytochemical evaluation in our study revealed phenols and tannins in methanol extract of *S. divinum* and only phenols, tannins and saponins in methanol extract of *S. girgensohnii*. Alkaloids and flavonoids were absent in methanol extract of both mosses (Table 1).

In the present investigation, the total phenolic content was observed in *S. divinum* (50.00 mg GAE/g) and *S. gingensohnii* (52.50 mg GAE/g) (Table 2). The methanolic extract of the studied bryophytes showed a high total phenolic content. Total phenolic content is an indicator of antioxidant potential and phenolic compounds in plants are thought to be responsible for free radical scavenging activity. The phenolic concentration in the methanol extract of *S. girgensohnii* was higher than in the methanol extract of *S. divinum*. However, the difference was not statistically significant. Chobot et al. (2006); reported a very low total phenolic content in the bryophyte *D. scoparium* (3.8%). In a study by Karaoğlu et al. (2022); the antioxidant activity of methanol extract

of *D. scoparium* was determined by DPPH free radical scavenging method and the total flavonoid and total phenolic content was calculated as 289.43µg/mL and 133.98 mg/g, respectively (DPPH; 38.98%). The researchers stated these data were higher than those reported in previous studies on the total phenolic content of *D. scoparium* extracts (Salvaroğlu et al., 2018; Bhadauriya et al., 2018). They suggested that geographical and environmental factors may have contributed to this difference. Preliminary phytochemical screening with the testreagentsfor the different extracts (petroleum ether, acetone, methanol, chloroform, and ethanol) of *D. scoparium* have been mentioned along with total phenolic content (45.42mg/g GAE at the concentration of 60µg/mL). In *Bryum capillare* Hedw., the total phenolic content was found to be 23.26 mg/g (Onbaşlı and Yuvalı, 2021). Some reports show higher and lower total phenolic content in mosses (Karim et al. 2014, Önder et al., 2021). Bhattari et al. (2008) reported that the extracts got from *S. uncinata* had high antioxidant activity, free radical scavenging activity, reducing power, superoxide radical scavenging activity, and ABTS [2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] cation scavenging activity. Another study on the extracts of *Polytrichastrum alpinum* showed that the extracts of the isolated compounds have a top level of antioxidant activity (Bhattarai, et al, 2009).

The results of our study are in agreement with the results obtained in other studies carried out on this subject. A high total phenolic content was found in the methanol extract of the bryophytes studied. Good antioxidant potential is indicated by high total phenolic content. Phenolic compounds in plants are thought to be responsible for important free radical scavenging activity. The polarity of the solvent is an essential factor in enhancing phenolic solubility (Naczk and Shahidi, 2006).

The DPPH radical assay is important because of its reaction mechanisms. Quantitative responses are altered by many environmental factors and the radical site is highly hindered to be easily accessible by complex molecules (Baliyan et al, 2022). Due to its stability and ease of use, this assay is commonly used for rapid screening of antioxidant capacity. In the present work, the antioxidant activities of the extracts were evaluated using the DPPH assay. In the present study, the results of the antioxidant potential of the methanolic extracts of *S. divinum* and *S. girgensohnii* obtained at different concentrations (10-100 µg mL^{-1}) are given in Table 3. The results of the experiments show that all concentrations of the methanolic extracts of *S. divinum* and *S.girgensohnii* have a DPPH scavenging activity higher than 50%. An increase in DPPH scavenging ability was observed with increasing concentration of the extracts. At each concentration tested, the DPPH scavenging ability of *S. divinum* methanol extract was higher than that of *S. girgensohnii* methanol extract. The results obtained in this study indicate that the *S. divinum* methanol extracts are radical scavengers and can react with the DPPH radical, which could be attributed to their electron donating ability. The antioxidant activity results of this research reveal that phenol and tannin in *S. divinum* and *S. girgensohnii* methanol extracts, and saponin compounds additionally found in *S. girgensohnii* methanol extract have quite high DPPH radical scavenging activity and total antioxidant capacity, but no cupric ion reducing activity. Pigment production is an important criterion for increasing antioxidant capacity. Our results support this state for *S. divinum* with dark reddish burgundy color.

Plant phenolics are the main group of compounds that act primarily as antioxidants or free radical scavengers. Antioxidant activity is correlated with total phenolic content. Therefore, it is a reasonable practice to determine the total phenolic content in herbal extracts. The results show that the total phenolic content of methanol extracts of *S. divinum* and *S. girgensohnii* is high. The radical scavenging activity is correspondingly high. However, phenolics alone do not determine antioxidant activity. Extracts with high antioxidant activity have high phenolic content (Kumbhare et al, 2012). Previous studies have shown that there is a significant linear relationship between the total amount of phenolic compounds and antioxidant activity in the foods and plant species analysed (Karakaya et al., 1999; Ivanova et al., 2005). The relationship between the structure of polyphenolic compounds and their antioxidant activity has been demonstrated. Monophenols are known to have lower antioxidant activity than polyphenols (Sanchez-Moreno et al, 1998). The results show that there is a positive linear relationship between the total phenolic content and the DPPH radical scavenging activity and the total antioxidant capacity of the *S. divinum* and *S. girgensohnii* methanol extracts. The CUPRAC test results show that the antioxidant compounds found in *S. divinum* and *S. girgensohnii* methanol extracts do not have a thiol group. On the other hand, phenolic compounds (phenols, tannins and anthraquinones) found in *S. divinum* and *S. girgensohnii* methanol extracts can be an excellent source for radical scavenging antioxidants, although they show very low Cupric ion reducing activity.

5. Conclusion

Türkiye has a significant history of folk medicine and in recent years, researchers have conducted many studies on traditional medicine and medicinal plants in Türkiye. Studies have shown that bryophytes can be rich in secondary metabolites such as alkaloids, flavonoids, carbohydrates, terpenoids, tannins, and phenolic substances and have antioxidant, anti-cancer, and anti-microbial properties. Phytochemical analyses and investigation of the biological properties of bryophytes are important for the production of new drugs to treat various diseases.

Previous studies and the results of our study show that bryophytes have high antioxidant content and are very promising in this respect. The results from the present work provided a new framework for the utilisation of the *S. divinum* and *S. girgensohnii* as a natural source of bioactive agents, such as antioxidants.

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Competing Interests

Authors have declared that no competing interests exist.

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