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Determination of Antioxidant Activity and Biochemical Content of *Homalothecium philippeanum* (Spruce) Schimp.

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Abstract

Living organisms produce reactive oxygen species (ROS) during the oxygen processing. ROS damage biomolecules and causes oxidative stress. Antioxidants prevent cellular damage against these harmful effects of ROS by neutralizing free radicals. Medicinal plants provide a rich source of antioxidants to reduce oxidative stress and play an important role in the treatment of diseases. Bioactive compounds, especially polyphenols and flavonoids, protect cells against oxidative damage by neutralizing free radicals. Bryophytes, especially mosses, are one of the plant groups that attract attention in this area. Mosses show biologically active properties with secondary metabolites such as terpenes and flavonoids they contain. In this study, extraction of *Homalothecium philippeanum* moss with ethanol, methanol, and n-hexane solvents was carried out and the biochemical content analysis of the extracts was investigated. 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester was detected as the major component in the ethanol and methanol extracts of the *H. philippeanum*. Biologically active alkanes such as Tetradecane and Hexadecane were found in the n-hexane extract. The antioxidant activity of moss ethanol extract was investigated using the DPPH method and the EC_{50} value was determined as 7.084 mg/ml.

Keywords: Bryophytes, *Homalothecium philippeanum*, Antioxidant Activity, Biochemical Content, GC-MS

Homalothecium philippeanum (Spruce) Schimp.'in Antioksidan Aktivitesinin ve Biyokimyasal İçeriğinin Belirlenmesi

Öz

Canlı organizmalar, oksijen işleme sürecinde, reaktif oksijen türleri (ROS) üretmektedirler. ROS'lar, biyomoleküllere zarar vererek oksidatif strese neden olmaktadır. Antioksidanlar, serbest radikalleri nötralize ederek ROS'un bu zararlarına karşın hücresel hasarı önlemektedir. Tıbbi bitkiler, oksidatif stresi azaltmak için zengin bir antioksidan kaynağı sunmakta ve hastalıkların tedavisinde önemli bir rol oynamaktadır. Özellikle polifenoller ve flavonoidler gibi biyoaktif bileşikler, serbest radikalleri etkisiz hale getirerek oksidatif hasara karşı hücreleri korur. Briyofitler, özellikle karayosunları, bu alanda dikkat çeken bitki gruplarından olmuşlardır. İçerdikleri terpenler, flavonoidler gibi sekonder metabolitlerle biyolojik olarak aktif özellikler göstermektedirler. Bu çalışma kapsamında, *Homalothecium philippeanum* karayosununun etanol, metanol ve n-hekzan çözücüleriyle ekstraksiyonu gerçekleştirilmiş ve ekstraktların biyokimyasal içerik analizi araştırılmıştır. Bitkinin, etanol ve metanol ekstraktlarında majör bileşen olarak 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester tespit edilmiştir. N-hekzan ekstraktında Tetradekan ve Hekzadekan gibi biyolojik olarak aktif alkanlar bulunmuştur. Karayosunu etanol ekstraktının antioksidan aktivitesi DPPH yöntemi kullanılarak araştırılmış ve EC_{50} değeri 7.084 mg/ml olarak belirlenmiştir.

Anahtar kelimeler: Briyofitler, *Homalothecium philippeanum,* Antioksidan Aktivite, Biyokimyasal İçerik, GC-MS

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1. Introduction

As living organisms process oxygen through enzymatic reactions, reactive oxygen species (ROS) emerge as by-products; these are reactive molecules capable of oxidizing proteins and lipids (Tretter et al., 2021). These reactive molecules play a critical role in biological processes such as oxidative stress and cellular damage (Hernansanz-Agustín & Enríquez, 2021). Due to the high reactivity of ROS and their independent production in cellular compartments, ROS levels are tightly regulated to prevent unwanted oxidation (Mittler et al., 2022). Molecules that inhibit free radicals and thus delay the damage occurring in cells are known as antioxidants (Nimse & Pal, 2015). Antioxidants scavenge reactive oxygen species (ROS), reducing damage to enzymes, proteins, DNA, and lipids caused by oxidative stress. Due to these protective properties, antioxidants play an important role in preventing chronic diseases (Miguel, 2011).

Extracts obtained from natural sources contain numerous secondary metabolites that play roles in organisms' defense mechanisms. These metabolites can be derived from plant tissues, microbial fermentation, and marine organisms (Mushtaq et al., 2018). Plants with natural antibacterial properties hold great potential as alternative treatment methods (AlSheikh et al., 2020). Numerous studies have shown that antioxidants play a significant role in maintaining human health, preventing diseases, and contributing to their treatment due to their ability to reduce oxidative stress. Therefore, measuring the antioxidant capacity of foods and biological samples is important to investigate their efficacy in the prevention and treatment of diseases associated with oxidative stress (Munteanu & Apetrei, 2021).

Medicinal plants have been used for therapeutic purposes throughout history. Today, most drugs are derived from natural products or their derivatives, and nearly 40% of drugs approved by the Food and Drug Administration (FDA) are of natural origin (Boy et al., 2018; Ursavaş and Tuttu, 2017; Çizgen et al. 2018a). Additionally, medicinal plants provide a rich source of natural antioxidants. Various studies have been conducted for years to find effective and safe antioxidants. Although *in vitro* studies yield promising results, a few natural and synthetic antioxidants have been developed for clinical applications due to their low efficacy and side effects. The belief that natural antioxidants are better and safer than synthetic ones has drawn attention to natural products for the development of new antioxidants (Tuttu and Ursavaş, 2017). It is hypothesized that plants, which produce ROS as by-products during the photosynthetic process, have a defense system composed of secondary metabolic products to protect themselves from oxidative damage (Na et al., 2011; Tuttu et al., 2017).

In mosses, the major groups of biologically active secondary metabolites are terpenes, steroids, cyanoglycosides, and various aromatic (phenolic) compounds. The second most comprehensive group is represented by flavonoids and their glycosides, bibenzyl and bis(bibenzyl) derivatives, alkyl and aryl benzoates, coumarins, and monomeric aromatic acids (Faleva et al., 2022). Polyphenols are the primary plant components with antioxidant effects. They are known for their redox abilities, such as the adsorption and neutralization of free radicals and the decomposition of peroxides. Flavonoids, which are common in plants, directly scavenge free radicals and inhibit enzymes involved in the formation of these radicals, thereby preventing oxidative damage (Stanković et al., 2016).

In this study, ethanol, methanol, and n-hexane extracts of the moss *H. philippeanum* were prepared, and the biochemical content analysis of each extract was performed. The aim of the biochemical content analysis was to examine and compare the diversity of biochemical components present in the extracts. Gas Chromatography-Mass Spectrometry (GC-MS) was used for these analyses. Ethanol extract of *H. philippeanum* was used in antioxidant activity test by DPPH method. This study is the first comprehensive research that determines the antioxidant activity and biochemical content analysis of *H. philippeanum* extracts. The fact that these analyses have not been previously applied to *H. philippeanum* highlights the novelty and significance of this study.

2. Materials and methods

2.1. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, ethanol, methanol, and n-hexane from Merck and ascorbic acid were purchased from Carlo Erba.

2.2. Collection localities

The moss *H. philippeanum* was collected and identified by Prof. Dr. Kerem CANLI from Akdağ in Amasya province, Turkey (N 40° 48' 4.8" E 36° 7' 52.6") on April 9, 2023. After collection, the sample, while still alive, was placed in a sample bag and transported to the laboratory, where it was air-dried at room temperature. It was then stored at the Fauna and Flora Research and Application Center (FAMER) herbarium at Dokuz Eylül University in Buca, Izmir, Turkey, until the experiments were conducted (Herbarium no: CANLI 0204). Due to

their poikilohydric nature, mosses can survive by losing water and minimizing their metabolic activity during dry periods (Shibata et al. 2018). The *H. philippeanum* sample was thus preserved in a way that allows it to revive quickly when re-exposed to water.

2.3. Preparation of extracts from Homalothecium philippeanum

For the extraction of active compounds from *H. philippeanum*, the moss sample was ground until it reached a powdered form. From the ground moss sample, 5 grams of each were transferred into Erlenmeyer flasks containing 200 milliliters of ethanol, methanol, and n-hexane solvents, respectively. The extraction of active compounds was carried out by shaking at 160 rpm at room temperature for 3 days. The extracts were then filtered and evaporated at 35-40°C under vacuum using Rotavapor R100 (Buchi Labortechnik AG, Switzerland). After the extraction, 0.017 g, 0.005 g, and 0.052 g of dry mass were obtained, respectively (Altuner et al., 2014).

The concentration of the moss extract prepared with ethanol was set to 1 mg/ml for use in the antioxidant activity test. The concentration of ascorbic acid, which was used as a positive control in the antioxidant test, was also prepared at 1 mg/ml.

2.4. Biochemical screening

Before GC-MS analysis, moss extracts were filtered through 0.45 µm injection filters to remove any residual particles. Biochemical tests were conducted following the methods described in Canlı et al. (2023). The GC-MS analyses were carried out using Agilent GC 8890 and Agilent GC/MSD 5977B instruments (Agilent Technologies Inc., USA). Helium served as the carrier gas, and retention times were determined by comparing them with the Wiley-Nist MS database. Chemical compounds present at concentrations above 0.5% were classified as major constituents. To ensure reliability, the analyses were repeated, and certain parameters were adjusted based on the solvents used.

2.5. Determination of antioxidant activity

The free radical scavenging capacity of *H. philippeanum* extract prepared with ethanol was assessed based on the ability of stable DPPH to absorb. This method is based on the fact that the dark purple color of the DPPH solution, detected at 515 nm, turns yellow as a result of the antioxidant molecules neutralizing stationary free DPPH radicals (Kedare and Singh, 2011). 0.0039 g of DPPH chemical was dissolved in ethanol. It was loaded into a microplate with 96 wells so that the concentrations of DPPH solution and moss extract were from 7.8125 to 1000 µg/ml. The microplate was kept in a dark environment at room temperature for 30 minutes. Following the incubation period, absorbance at 515 nm was measured using a microplate reader. Ascorbic acid was used as positive control in this assay, and all experiments were performed in triplicate.

2.6. Statistical analysis

The results obtained from three independent repetitions for each antioxidant activity are expressed as mean ± standard deviation (SD). Following statistical analysis of the data, EC_{50} values were determined using Four-Parameter Logistic Regression with a 95% confidence interval (Chen et al., 2013). Data were analyzed using One-Way ANOVA (Analysis of Variance) and Pearson correlation tests in R Studio (version 2024.09.0). The level of statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Biochemicals in extracts

The area % covered by the substances identified through GC-MS analysis are listed in Table 1.

Table 1. Biochemical screening of *H. philippeanum*

Classification	Compound name	RT	RI	Formula	MW (g/mol)	HP Ethanol extract	HP Methanol extract	HP n-Hexane extract	Known activity
Alcohols	1,3-Pentanediol, 2,2,4-trimethyl-	9.815	1157	$C_8H_{18}O_2$	146.23	2,42	-	-	-
	Ethanol, 2-(2-butoxyethoxy)-	10.325	1169	$C_8H_{18}O_3$	162.23	-	1,17	-	-
	Lauryl alcohol	14.156	1457	$C_{12}H_{26}O$	186.33	1,56	-	-	-
	Phytol Isomer	20.982	2104	$C_{20}H_{40}O$	296.5	-	1,34	-	Antimicrobial, antioxidant and anti-inflammatory activity (Edewor et al., 2016)
	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	30.059	1653	$C_{15}H_{26}O$	222.37	-	4,64	10,71	Antimicrobial, antioxidant and anti-inflammatory activity (Illozue et al., 2024)
Alkanes	Dodecane, 2,6,11-trimethyl-	11.533	1257	$C_{15}H_{32}$	212.41	-	-	1,82	-
	Tetradecane	13.157	236	$C_{14}H_{30}$	198.39	-	-	10,44	Antibacterial and antifungal activity (Nasr et al., 2022)
	Cyclododecane	14.157	1316	$C_{12}H_{24}$	168.32	-	2,32	-	-
	Tetracosane	14.386	366	$C_{24}H_{50}$	338.7	-	-	2,90	Antibacterial and antioxidant activity (Asha et al., 2017)
	Hexadecane	15.628	268	$C_{16}H_{34}$	226.44	-	-	4,64	Antibacterial, antifungal and antioxidant activity (Nepal et al., 2021)
	Dodecane	14.933	200	$C_{12}H_{26}$	170.33	-	-	2,73	Antibacterial activity and enhances antifungal activity (Stopiglia et al., 2012; Octarya et al., 2021)
	Hexadecane, 2,6,10,14-tetramethyl-	16.873	1811	$C_{20}H_{42}$	282.5	-	-	8,56	-
	Octadecane	17.844	296	$C_{18}H_{38}$	254.5	0,66	1,09	4,55	-
	Nonadecane	18.876	312	$C_{19}H_{40}$	268.5	1,27	0,96	2,71	-
	Tetratriacontane	19.143	3400	$C_{34}H_{70}$	478.9	-	-	2,31	Antimicrobial activity (Sumerta et al., 2022)
	Heptacosane	19.556	426	$C_{27}H_{56}$	380.7	-	-	1,66	Antioxidant activity (Akpuaka et al., 2013)
	Eicosane	19.860	345	$C_{20}H_{42}$	282.5	2,07	1,57	5,58	Antibacterial and antifungal activity (Octarya et al., 2021)
	Heneicosane	20.800	342	$C_{21}H_{44}$	296.6	1,53	-	3,26	Antimicrobial activity (Kumosani et al., 2024)
	Docosane	21.698	356	$C_{22}H_{46}$	310.6	1,14	0,98	3,47	Antibacterial activity (Akpuaka et al., 2013)
	Tricosane	21.705	370	$C_{23}H_{48}$	324.6	-	-	4,26	Antimicrobial activity (Baltacı et al., 2022)
Alkenes	1-Tetradecene	13.055	1385	$C_{14}H_{28}$	196.37	0,98	-	-	Antimicrobial activity (Naragani et al., 2016)
	1-Hexadecene	15.547	1592	$C_{16}H_{32}$	224.42	1,32	-	-	Antibacterial activity (Egbung et al., 2017)
	Neophytadiene	18.275	1827	$C_{20}H_{38}$	278.5	1,01	1,01	-	Anti-inflammatory and antimicrobial activity (Nepal et al., 2021)
Carboxylic Acids	Hexanoic acid	7.473	973	$C_6H_{12}O_2$	116.16	-	3,23	-	-
	Hexanoic acid, 2-ethyl-	9.541	1116	$C_8H_{16}O_2$	144.21	-	2,01	-	-
	Nonanoic acid	11.653	1268	$C_9H_{18}O_2$	158.24	-	2,15	-	Antifungal activity (Jang et al., 2012)

Esters	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	12.690	1351	$C_{12}H_{24}O_3$	216.32	13,35	-	-	-
	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	12.961	1365	$C_{12}H_{24}O_3$	216.32	11,39	9,47	-	-
	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	15.689	1591	$C_{16}H_{30}O_4$	286.41	19,27	16,01	7,47	-
	Carbonic acid, di(decyl) ester	16.547	2328	$C_{21}H_{42}O_3$	342.6	-	-	1,70	-
	1-(4-Isopropylphenyl)-2-Methylpropyl acetate	16.591	1800-2000	$C_{15}H_{22}O_2$	234.33	1,49	0,83	2,25	-
	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	18.692	1819	$C_{16}H_{22}O_4$	278.34	21,76	17,82	2,24	Antifungal, antioxidant and antibacterail activity (Asha et al., 2017; Lanchana & Garampalli, 2024)
	Hexadecanoic acid, methyl ester	19.162	1908	$C_{17}H_{34}O_2$	270.5	-	2,60	-	Antioxidant and antibacterial activity (Edewor et al., 2016; Shaaban, et al., 2021)
	Dibutyl phthalate	19.621	1909	$C_{16}H_{22}O_4$	278.34	3,06	2,42	-	-
Ketones	2-Pentadecanone, 6,10,14-trimethyl	18.359	1842	$C_{18}H_{36}O$	268.5	1,25	1,04	-	Antimicrobial activity (Amos-Tautua et al., 2020)
Linoleic Acids	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	20.874	-	$C_{19}H_{32}O_2$	292.5	-	2,19	-	Anti-inflammatory, antioxidant and antimicrobial activity (Edewor et al., 2016; Akpuaka et al., 2013)
Others	Unknown	9.469	-	-	-	1,96	-	-	-
	2,2-dimethyl-3-methylene-bicyclo [2.2.1] heptane	10.407	-	$C_{14}H_{20}O_4$	252.31	0,91	-	-	-
	Unknown	12.700	-	-	-	-	10,86	-	-
	Unknown	13.241	-	-	-	1,48	-	-	-
	Unknown	13.243	-	-	-	-	-	1,92	-
	Unknown	13.245	-	-	-	-	0,77	-	-
	Unknown	16.868	-	-	-	3,39	-	-	-
	Unknown	16.872	-	-	-	-	2,60	-	-
	Unknown	17.959	-	-	-	2,34	-	-	-
	Unknown	17.960	-	-	-	-	1,92	-	-
	Unknown	17.964	-	-	-	-	-	3,88	-
Phenols	2,4-Di-tert-butylphenol	14.700	1519	$C_{14}H_{22}O$	206.32	-	-	5,02	Antioxidant, antimicrobial and antifungal activity (Nepal et al., 2021; Zou et al., 2023)
	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	23.703	2365	$C_{23}H_{32}O_2$	340.5	-	0,95	4,18	Antifungal activity (Hameed & AL-Muhsin., 2024)
RT: Retention time, RI: Retention Index, MW: Molecular Weight, HP: *Homalothecium philippeanum*, "- "Activity not researched; http://www.chemspider.com/; https://pubchem.ncbi.nlm.nih.gov/; https://comptox.epa.gov/									

3.2. DPPH scavenging activity

H. philippeanum moss was extracted with different solvents and only the antioxidant capacity of the ethanol extract was examined. Antioxidant capacity was assessed based on the ability to neutralize DPPH radicals. The antioxidant capacities of *H. philippeanum* and ascorbic acid at concentrations ranging from 7.813 to 1000 µg/ml are presented in Table 2. Results indicate that the ethanol extract of *H. philippeanum* scavenged DPPH radicals by 25% to 49% within this concentration range. Increased extract concentration led to enhanced DPPH radical scavenging. Ascorbic acid, used as a positive control at 1000 µg/ml, demonstrated approximately 95% DPPH scavenging capacity. The EC_{50} values were determined as 7.084 mg/ml for *H. philippeanum* extract and 0.04 mg/ml for ascorbic acid. The correlation between the DPPH scavenging activities of the extract and ascorbic acid was analyzed, with a correlation coefficient of 0.904849. Given the p-value ($p < 0.05$), the results were considered statistically significant.

Table 2. Results of the DPPH radical scavenging activity of *H. philippeanum* ethanol extract and ascorbic acid (%) with mean ± standard deviation.

Concentration (µg/ml)	***H. philippeanum***	**Ascorbic acid**
1000	48.72 ± 0.88	94.71 ± 0.00
500	41.28 ± 0.95	94.33 ± 0.06
250	37.00 ± 0.89	92.43 ± 0.01
125	34.93 ± 1.21	91.01 ± 0.02
62.5	34.44 ± 2.82	73.04 ± 0.04
31.25	33.68 ± 1.48	40.23 ± 0.07
15.625	30.14 ± 2.48	23.21 ± 0.27
7.81	25.83 ± 0.25	11.77 ± 0.04

4. Discussion and Conclusion

Plants produce a variety of secondary metabolites (phytochemicals), in addition to primary metabolites, which play roles in interspecies interactions (Çizgen et al., 2018b; Süntar, 2020). Secondary metabolites like alkaloids, terpenoids, and phenolics protect plants from microbial infections, with phenolic compounds standing out due to their high biological activity. These compounds exhibit antimicrobial and antioxidant properties, making it difficult for pathogens to develop resistance. Thus, plant-based antimicrobials show great potential in the search for new biopharmaceutical products (Martelli & Giacomini, 2018). Some studies in the literature showing that bryophytes produce numerous secondary metabolites responsible for antitumor, antifungal, antioxidant, and antimicrobial activities (Bandyopadhyay & Dey, 2022; Benek et al., 2022; Cianciullo et al., 2021). A limited number of findings have been obtained in literature on the *H. philippeanum*. Therefore, three different extractions of *H. philippeanum* moss were conducted using ethanol, methanol, and n-hexane solvents. The maceration method was chosen for extraction under conditions detailed in the materials and methods section. This study aims to determine the antioxidant activities and biochemical content of these extracts.

The antioxidant activity test results indicate that the antioxidant capacity of the ethanol extract of *H. philippeanum* is lower compared to ascorbic acid. While ascorbic acid reaches a half-maximal effect at much lower concentrations, *H. philippeanum* extract requires a higher concentration to achieve a similar effect. Within the concentration range tested, ascorbic acid exhibited lower antioxidant activity than *H. philippeanum* at the two lowest concentrations, 0.0078 and 0.0156 mg/ml. Despite the strong positive correlation indicated by the correlation coefficient ($r = 0.904849$), this observation suggests the possibility of some deviations at low concentrations. Although there is a high correlation between the extract and the positive control, indicating a general parallelism, the higher activity of *H. philippeanum* extract at lower concentrations suggests that the compounds it contains may have different mechanisms of action at specific concentrations.

Another study presented in the literature also focused on the antioxidant properties of *Homalothecium sericeum*, belonging to the same genus as *H. philippeanum*. Sahilli et al. (2018) carried out a study on *H. sericeum*, wherein this species was recorded to possess high antioxidant activity too. These findings denote the congruence between the results of the two studies, which hint that members of the same genus may potentially possess antioxidant activity.

In research in literature, the antimicrobial activities of the methanol extract of *H. philippeanum* have

been studied. This study, conducted by Veljić et al. (2008), reveals that the methanol extract of the *H. philippeanum* exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. *S. aureus* is a pathogen associated with hospital infections due to the enterotoxins it produces, while *E. coli* causes urinary tract and organ infections (Cui et al., 2019; Wang et al., 2020). The activity detected against these two public health-threatening bacteria has envisaged further investigation of the biological activity of *H. philippeanum*.

The GC-MS analysis of *H. philippeanum* confirmed the presence of various compounds with biological activities, including antimicrobial, antioxidant, and antifungal properties. 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (21.76%), which is determined as the major component in ethanol extract, is a compound known for its biological properties such as antioxidant, antifungal, and antibacterial (Asha et al., 2017; Lanchana & Garampalli, 2024). The Alkane type compounds Eicosane (2.07%) and Docosane (1.14%) are known for their antibacterial effects and Heneicosane (1.53%) has been reported in the literature to have antimicrobial activity (Akpuaka et al., 2013; Octarya et al., 2021; Kumosani et al., 2024). Another compound with known antimicrobial activity, 2-Pentadecanone, 6,10,14-trimethyl (1.25%), was identified in the extract (Amos-Tautua et al., 2020).

1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (17.82%), which is considered a major substance and has more than one biological activity, were detected in the methanol extract of the plant, just like in the ethanol extract. Nonanoic acid (2.15%), one of some compounds proven to show antifungal activity, was determined in the extract (Jang et al., 2012). Hexadecanoic acid, methyl ester (2.60%), one of the esters observed in methanol extract, is another compound with proven antioxidant and antibacterial effects (Edewor et al., 2016; Shaaban, et al., 2021). 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (4.64%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- (2.19%) and Phytol Isomer (1.3%) compounds are known for their anti-inflammatory activities in addition to their antimicrobial and antioxidant effects (Akpuaka et al., 2013; Edewor et al., 2016; Illozue et al., 2024).

In the n-hexane extract of the plant, most identified compounds consist of alkanes known for their biological activities. The major compound, Tetradecane (10.44%), is an alkane with known antibacterial and antifungal activity (Nasr et al., 2022). Hexadecane (4.64%), in addition to the known activities of Tetradecane, also has an antioxidant effect (Nepal et al., 2021). Tetratriacontane (2.31%), Heneicosane (3.26%), and Tricosane (4.26%) are alkane compounds known solely for their antimicrobial activities (Baltacı et al., 2022; Sumerta et al., 2022; Kumosani et al., 2024). While Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (4.18%) has proven antifungal activity, 2,4-Di-tert-butylphenol (5.02%) is recognized not only for its antifungal effects but also for its antimicrobial and antioxidant properties (Nepal et al., 2021; Zou et al., 2023; Hameed & AL-Muhsin, 2024).

Some of the compounds identified through biochemical content analysis were detected in all three extracts, while the majority were observed only in one extract. Extraction yield is influenced by the solvent used and the chosen extraction method (Sultana et al., 2009). In a study conducted by Değirmenci and Ezer (2024), supercritical CO_2 extraction of *H. philippeanum* moss was performed to assess its effect on enzyme activity, and various bioactive compounds were identified via GC-MS analysis. In the mentioned study, ethanol was used as cosolvent, and solvent usage was minimized by supercritical extraction. Bioactive compounds such as 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- and Tetracosane were detected in both maceration and supercritical extracts and these two compounds were found in higher amounts in GC-MS analysis of maceration extracts. When comparing extraction methods, supercritical CO_2 extraction can be insufficient for detecting compounds with specific polarities, whereas solvent-based extractions yield a broader range of compounds.

In the GC-MS analysis of *H. philippeanum*, compounds with various biological activities were obtained using ethanol, methanol, and n-hexane solvents. While ethanol and methanol extract present compounds from various classes, the n-hexane extract contains more compounds with biological activity. Notably, n-hexane effectively extracted compounds with antimicrobial, antifungal, and antioxidant effects, such as alkanes and phenols. Ethanol and methanol, on the other hand, were more effective in extracting polar compounds. Solvent polarity has a direct impact on the diversity and biological activity of the compounds obtained, making acareful solvent selection is essential in phytochemical extraction strategies. Therefore, solvent selection is of great importance to maximize detection of biological activity.

This research represents one of the first antioxidant studies conducted on *Homalothecium philippeanum*. This study is an important step towards discovering the potential biological activities of the plant and was supported by a GC-MS analysis, especially evaluating its antioxidant properties in this context, it was aimed to develop an in-depth understanding of the nature and quantity of phytochemical components contained in *H. philippeanum*. The analysis in question determined the chemical profile of the plant and revealed the presence of components that promote antioxidant activity. The data obtained highlights the potential health benefits of this plant while also providing a basis for future studies.

Declaration

Author contributions

Idea/Concept: SDB, DT, KC; Conceptualization and design: SDB, DT, AB; Auditing consulting: AB, KC; References: KC; Materials: GG, CY; Data collection and/or processing: SDB, DT, GG, AB; Analysis and/or interpretation: SDB, DT, CY; Literature search: SDB, KC, CY, GG; Writing phase: SDB, DT; Critical review: AB, KC.

Conflict of interest

The authors declare that there is no conflict of interest related to the content of this study.

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Ethical approval

This research does not involve human or animal subjects; therefore, ethics approval is not required.

Additional information

This study was partly presented as a poster at the 6th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2023) by Bozkurt et al. (2023). The abstract of that poster is available in the full-text proceedings on page 1521 of the 6th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2023). These results are discussed in this article in the context of a broader perspective.

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