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TOTAL PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF MOSSES FROM YENICE FOREST (IDA MOUNTAIN)

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ABSTRACT:

Recent pharmacological investigations of bryophytes have proven that the active principles present in these plants are quite unique and having potential chemical application and antioxidant capacity. In this study, the volatile components in extracts from Thuidium tamariscinum (Hedw.) Schimp. and Platyhypnidium riparioides (Hedw.) Dixon, Kazdağları (Kalkim-Yenice, Çanakkale, Turkey) were isolated by solid phase micro extraction technique and identified by mass selective detector gas chromatography (GC-MS). Antioxidant capacities of these species were determined by CERAC and CUPRAC methods and phenolic contents by Folin-Ciocalteu method.

Keywords: *Thuidium tamariscinum and Platyhypnidium riparioides; mosses; antioxidant activity; GC-MS; TEAC, CUPRAC methods.*

1. INTRODUCTION

Plant phenolic are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Löliger, 1991). Most phytochemicals in natural agricultural sources have been generally recognized as bioactive or health-promoting compounds, which play an important role in

preventing cardiovascular diseases, cancers, obesity and diabetes, lowering blood cholesterol level, and reducing inflammatory action (Halliwell, 1996). Mosses and liverworts are small, low-growing plants and constitute the phylum Bryophyta, which is phylogenetically placed between vascular plants and algae. Bryophyta has more than 22.000 members all over the world, and nearly 3000 bryophytes are reported to have medicinal value and therefore, the members of this unique division in the plant kingdom are now increasingly used as new sources of pharmaceuticals. One interesting class of bryophytes, the liverworts are being therapeutically used worldwide, especially in Indian and Chinese cultures for the treatment of hepatitis and skin disorders due to their antibiotic, anti- inflammatory, and diuretic properties (Friederich, 1999; Gökbulut et al., 2012; Saroya, 2011). Bryophytes are considered as a "remarkable reservoir" of new, natural products or secondary compounds, many of which have shown interesting biologic activity. These activities can be presented as: antimicrobial, antifungal, cytotoxic, antitumor, vasopressin (VP) antagonist, cardiotonic, allergy causing, irritancy and tumor effecting, insect anti-feedant, insecticidal, molluscicidal, piscicidal, plant growth regulatory, superoxide anion radical release inhibition and 5-lipoxygenase, calmodulin, hyaluronidase, and cyclooxygenase inhibition features of bryophytes (Asakawa et al., 2013). Bryophytes are useful plants as sources of natural products since they grow everywhere in the world. The secondary metabolites identified from mosses belong to terpenoids, flavonoids and bibenzyls, but they are also rich in other compounds such as fatty acids, acetophenols and antimicrobial activity is related to the specific chemical composition, structural configuration of compounds, functional groups, as well as potential synergistic or antagonistic interactions between compounds.

Antioxidant capacity of the moss was found to be higher than certain common plants. High level of antioxidants present in liverworts and mosses can serve as a future source for medicinal and cosmetic purpose. Traditional medicinal use of bryophytes includes different ailments viz. inflammation, skin disease, wound healing (Singh et al., 2006), viral diseases (Frahm, 2004) etc. Bryophytes, especially liverworts, often have distinct odors, suggesting aromatic compounds such as phenols. However, few bryophytes have been linked to actual curative properties and identifiable associated compounds many antibiotics have been isolated from bryophytes, but few have been developed for medical use, despite their demonstrated effectiveness. In Germany, *Ceratodon purpureus* and *Bryum argenteum* are used to cure fungal infections of horses. Several medical uses seem promising, such as anti-leukemic properties and anticancer agents (Glime, 2013). In India, people of Kumaon Himalaya use *Marchantia polymorpha* and *M. palmata* to cure burns, abscesses and to reduce pus formation, while paste of Riccia spp. is applied on the ring worm disease of skin (Pant and Tewari, 1989; Kumar et al., 2000; Dhondiyal et al., 2103).The Gaddi tribes of Himachal Pradesh, India use *Plagiochasma appendiculatum* for the cure of burns, boils and blisters of skin (Kumar et al., 2000; Dhondiyal et al., 2103).The folklore use of bryophytes could be due to certain active compounds having antioxidant capacity. Ethno medicinal use of different bryophytes should be scientifically investigated for active principles in order to bridge between traditional knowledge and pharmacology (Yayintas et al, 2017).

The aim of this study was to investigate the antioxidant activity and volatile component of *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon.

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The present assay was designed to establish the volatile compounds of these mosses and their total antioxidant capacity (TAC), total phenolic content (TPC) using a combination of CERAC, CUPRAC, Folin-Ciocalteu methods.

Extraction of mosess

Water extraction: The dried mosses sample (0.25 g powdered of *Thuidium tamariscinum* or 0.50 g *Plathypnidium riparioides*) was pounded into small parts with a porcelain mortar. The dried powdered aerial parts of the plants were extracted with 50 mL boiling distilled water and were mixed with sonicate for 10 min and orbital shaker for 10 min at 450 rpm. The extract was filtered through a Whatman filter paper into a 50 mL-flask, and diluted to the mark with distilled water. The aqueous extract of bryophytes was prepared just before the experiments so as to prevent any undesired degradation reactions.

Methanol extraction: 0.25 g powdered of *Thuidium tamariscinum* and 0.50 g *Plathypnidium riparioides* mosess were used. These were extracted in stoppered flasks using 100 % (v/v) methanol. Two successive batch extractions were carried out using the stirrer. The first extraction was made with 30 mL 100 % MeOH for 60 min, the second with 20 mL 100 % MeOH for 60 min at 450 rpm. The two extracts were filtered and combined in a single graduated flask and diluted to 50 mL with 100 % MeOH at room temperature.

Gas chromatography-mass spectrometry (GC/MS)

Volatile compounds from moss samples were isolated by solid-phase microextraction (SPME) technique (Pawliszyn, 2012) and identified by Gas chromatography mass spectrometry. 0.2 g of moss sample was weighed in a 40-mL amber-coloured screw-top vial with a hole-cap polytetrafluoroethylene/silicon septum (Supelco, Bellefonte, USA), and 0.2 g of NaCl and 5 mL of distilled water were added to the vial. The vial was kept at 40 °C in a water bath for 20 min to equilibrate volatiles in the headspace. Then, an SPME (2 cm to 50/30 µm DVB/Carboxen/PDMS, Supelco, Bellefonte) needle was inserted into the vial. The SPME fibre was exposed at a depth of 2 cm in the headspace of the vial. Then, the SPME needle was immediately injected into a GCO or GC–MS column.

Volatiles were tentatively identified by GC–MS. A nonpolar HP5 column (30 m × 0.25 mm i.d. × 0.25-µm film thickness; J&W Scientific) was used for separation of volatiles. The GC–MS system consisted of an HP 6890 GC and 7895C mass-selective detector (MSD; Agilent Technologies, Wilmington, DE, USA). The GC oven temperature was programmed from 40 to 230 °C at a rate of 10 °C·min^{-1}, with the initial and final hold times of 5 and 20 min, respectively. Helium was used as a carrier gas at a flow rate of 1.2 mL·min^{-1}.The MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 35 to 350 amu; scan rate, 4.45 scans·s^{-1}. The identification of flavor compounds was based on comparison of the mass spectra of unknown compounds with those in the databases of the National Institute of Standards and Technology and Wiley Registry of Mass Spectral Data. Flavor compounds were quantified based on their relative abundances. (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany)

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CUPRAC spectrophotometric assay of total antioxidant capacity

1mL 10 mM cupric chloride, 1 mL 7.5mM neucuproine, 1 mL 1 M ammonium acetate buffer (pH:7) and 1 mL water were mixed. 0.1 mL bryophytes extracts were added in this mixture. The samples were incubated for half an hour at room temperature, absorbance against a reagent blank was measure at 450 nm. The results were expressed as mmol catechin per gram dry mosses (Apak et al.,2004).

CERAC spectrophotometric assay of total antioxidant capacity

1 mL of 2.0 x 10^{-3} M Ce(IV) solution + x mL of the bryophytes extract was placed into a test tube and diluted to 10 mL with H_2O. The mixture was allowed to stand for 30 min at room temperature and the 320-nm absorbance (A_{320} nm) was measured. The total antioxidant capacity was determined and results expressed as mmol equivalents dry weight (Ozyurt et al., 2007). The molar absorptivity of catechin in the CERAC method is $\varepsilon = 1.31\times104$ L mol^{-1} cm^{-1}. The results were expressed as mmol catechin per gram dry mosses (Ozyurt *et al*., 2007). The molar absorptivity of catechin in the CERAC method is $\varepsilon = 1.31\times104$ L mol^{-1} cm^{-1}. The results were expressed as mmol catechin per gram dry mosses.

Folin-Ciocalteu assay of total phenolic content

Samples were analyzed spectrophotometrically for contents of total phenolic by a *Folin–Ciocalteu* colorimetric method (Singleton et al., 1999). Volume of 1.5 mL of deionized water and 0.5 mL of the extract were added to a test tube, followed by addition of 2.5 mL of Lowry C solution was added, and the mixture was allowed to stand for 10 min. At the end of this period, 0.25 mL of Folin reagent was added, and 30 min were allowed for stabilization of the blue color formed. The molar absorptivity of gallic acid in the $AlCl_3$/ $NaNO_2$ colorimetric method is $\varepsilon = 5.14\times10^3$ L mol^{-1} cm^{-1}. The results were expressed as mmol gallic acid per gram dry mosses.

2. RESULTS AND DISCUSSION

GC-MS identification of water extracts

The mosses were sampled from Yenice forest from Ida Mountain (Kazdağ). They are abundant in nature. Data obtained from GC-MS from the sample of water extracts of *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon were given in Figure 1,2 and Table 1 respectively.

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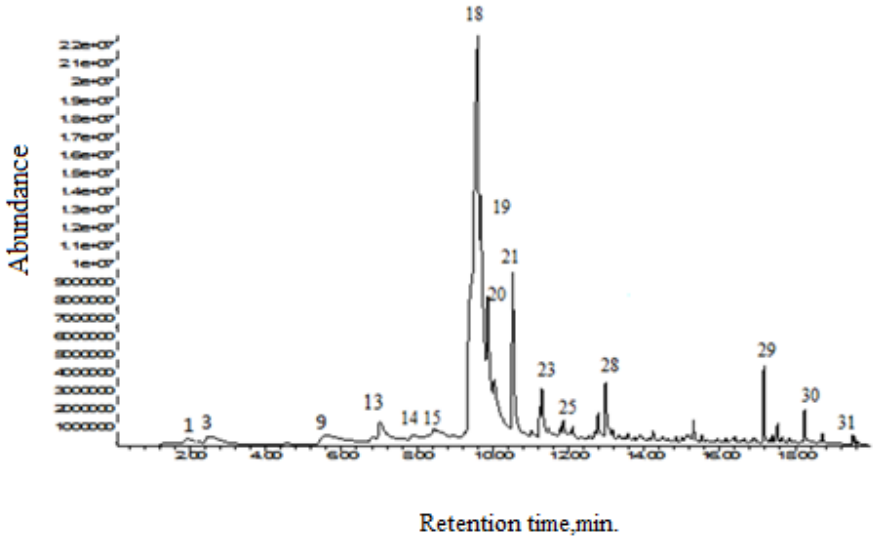

Figure 1. Mass spectrometry-gas chromatogram of the components of *Thuidium tamariscinum*; Number refer to the compounds given in Table 1.

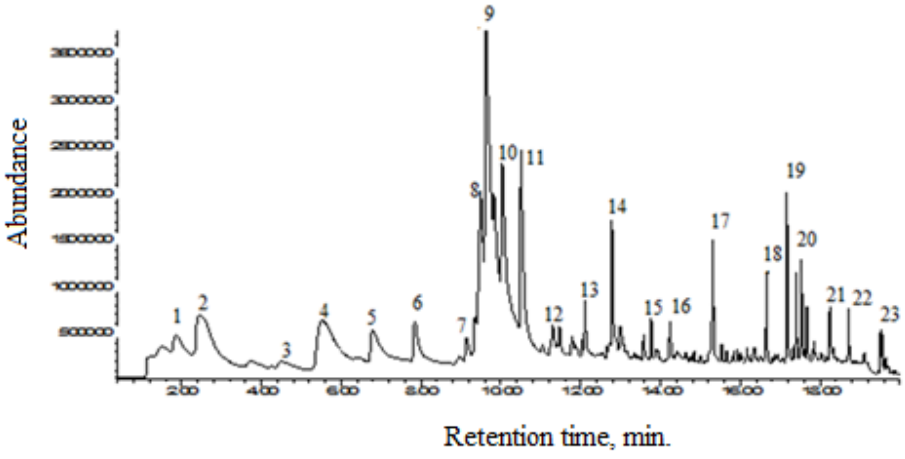

Figure 2. Mass spectrometry-gas chromatogram of the components of *Plathypnidium riparioides*; Number refer to the compounds given in Table 1.

A large number of compounds were determined from water extract of *Thuidium tamariscinum* and *Platyhypnidium riparioides* by GC-MS. Among these compounds with higher abundance were given in Table 1.

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Table 1. GC-MS analysis of *Thuidium tamariscinum* and *Plathypnidium riparioides* (water extract) component.

	Compound No	Retention time (min)	Component	Properties	Abundance %
Thuidium tamariscinum					
	1	1.93	Chloroform	plant volatile	1.00
	2	2.49	Trimethylsilyl trifluoroacetate		0.65
	3	2.63	Dimethyl-silanediol		1.36
	4	3.79	1-Pentanol	odor	0.35
	5	4.37	Benzenemethanol, .alpha.-(1-aminoethyl		0.09
	6	4.40	3-Methoxyamphetamine	stimulant drug	0.01
	7	4.54	Hexanal	used in the flavor industry to produce fruity flavors	0.48
	8	5.07	4-(2-Methylamino)ethyl)pyridine	nucleophilic catalyst	0.05
	9	5.16	2,3-Dimethylamphetamine	stimulant drug	0.10
	11	6.41	N-ethyl-1,3-dithioisoindoline	psychoactive drug	0.26
	12	6.82	Hexamethyl cyclotrisiloxane	polymeric, oil	0.61
	13	7.02	1,3-cis,5-cis-octatriene	herbal scent, plant defense and anti-fungal properties	2.73
	14	7.90	2-Amino-5-methylbenzoic acid		1.13
	15	8.44	2,4,6-Octatriene, all-E-		2.33
	16	8.67	1,2-dimethyl-1,4-cyclohexadiene	terpenoids	0.92
	17	8.95	1,2-Dimethyl-1,4-cyclohexadiene	mushroom alcohol	0.91
	18	9.60	1-Octen-3-ol	sharp, sweet odor reminiscent of butterscotch and acetone.	27.30
	19	9.69	Neopentyl ethyl ketone	organosilicon	5.59
	20	10.04	Octamethyl-cyclotetrasiloxane	fruit-like taste.	5.59
	21	10.53	2-Butenedioic acid (E)-, bis(2-	fatty oily sweet	5.65

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			ethylhexyl) ester	fruity	
	22	11.24	2-Octen-1-ol, (E)-	Food flavor/aroma component	0.65
	23	11.30	Bromoacetic acid, decyl ester	fruity, pungent odor	1.92
	24	11.48	Decamethyl-tetrasiloxane	slightly volatile	0.83
	25	11.80	Trifluoroacetic acid, 4-methylpentyl ester	sharp odor similar to vinegar	0.60
	26	11.87	Nonanal	A colorless, oily liquid, nonanal is a component of perfumes	0.90
	27	12.79	p-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate	acrid odor	1.12
	28	13.16	1-Dimethylthexylsilyloxybutane		0.57
	29	17.53	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane		0.52
	30	18.24	Ethylparaben	antifungal preservative. food additive	0.61
	31	19.51	Ethanedioic acid, bis(trimethylsilyl) ester		0.18

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			Table 1 continued		
	Compound No	**Retention time (min)** **(daki (min)**	**Component**	**Properties**	**Abundance %**
Plathypnidium riparioides	1	1.87	Chloroform	plant volatile	2.74
	2	2.45	Trimethylsilyl trifluoroacetate		6.49
	3	4.50	Hexanal		1.70
	4	5.54	2,3-Dimethylamphetamine		5.76
	5	6.78	Hexamethyl cyclotrisiloxane	polymeric, oil	2.91
	6	7.84	N-ethyl-1,3-dithioisoindoline		2.96
	7	8.97	1,2-Dimethyl-1,4-cyclohexadiene		0.70
	8	9.50	2,6,10,14-tetramethyl-pentadecane		3.75
	9	9.63	1-Octen-3-ol		8.29
	10	10.04	Octamethyl-cyclotetrasiloxane	organosilicon	7.56
	11	10.51	2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester		5.73
	12	11.30	Bromoacetic acid, decyl ester		1.2
	13	12.11	2,6,10,14-tetramethyl- Pentadecane		1.30
	14	12.99	p-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate		1.13
	15	13.99	2-Ethylacridine		0.36
	16	14.14	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)-		0.21
	17	15.31	Quinoline, 4-(4-chlorophenoxy)-8-fluoro-2-trifluoromethyl-		1.81
	18	16.93	Geosmin		0.26
	19	17.18	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-		1.47
	20	17.53	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane		0.93
	21	18.53	Quinoline, 4-(4-chlorophenoxy)-8-fluoro-2-trifluoromethyl-		0.16
	22	18.71	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester		0.67
	23	19.71	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl		0.15

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Determination of total antioxidant capacity and phenolic content

The total antioxidant capacity of mosses from methanol extracts and aqueous infusion determined by CERAC and CUPRAC methods. The results of the analysis calculated as mmol catechin/g dry mosses and given in Table 2 and Table 3. The phenolic content of mosses from methanol extracts and aqueous infusion determined by Folin- Ciocalteu method. The results of the analysis calculated as results are calculated as mmol gallic acid/g sample and given in Table 2 and Table3.

Table 2. The total amount of antioxidant capacity and phenolic content of mosses with 100 % (v/v) methanol

Samples	**CERAC (mmol catechin/ g mosses)**	**CUPRAC (mmol catechin / g mosess)**	**FOLIN- CIOCALTEU (mmol gallic asit/ g mosses)**
Thuidium tamariscinum	0.01200	0.00701	0.12900
Plathypnidium riparioides	0.00602	0.00327	0.06290

Table 3. The total amount of antioxidant capacity and phenolic content of aqueous infusion of mosses.

Samples	**CERAC (mmol catechin/ g mosses)**	**CUPRAC (mmol catechin / g mosess)**	**FOLIN- CIOCALTEU (mmol gallic asit/ g mosses)**
Thuidium tamariscinum	0.00403	0.00018	0.00632
Plathypnidium riparioides	0.00210	0.00039	0.00461

3. CONCLUSION

This study presents the results of a moss extracts of *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon were collected from Yenice forest to Ida Mountain (Kazdagı) Canakkale, Turkey, by chemical composition antioxidant activities.

These results are the proof that *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon extract possesses potent antioxidant activity. These significant proportions of the antioxidant activity are caused by phenolic substances in mosses structure. To sum up, here it was demonstrated that the extract of the mosses investigated here have great potential to be used in medicine, cosmetics and pharmaceutical applications as well as food and agricultural use.

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