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Screening of antimicrobial, cytotoxic effects and phenolic compounds of the moss *Dicranum scoparium*

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Abstract

Bryophytes belong to the group of the oldest known land plants, which includes liverworts, hornworts, and mosses. Recent research shows that mosses contain remarkable and unique substances with high biological activity. This study describes phenolic compounds, antimicrobial and cytotoxic activities of *Dicranum scoparium* (Hedw.) collected from Sundiken Mountains, Eskisehir. Total phenols contents of the extracts were determined by Folin-Ciocalteu assay and their amount ranged from 81.90± 0.10 to 105.21± 0.10 mg/g of gallic acid equivalents. The highest total phenolic content were obtained from the ethyl acetate extract (extract C). Phenolic acids in the extracts acquired by using different polarity solvents were separated by reverse-phase High-Performance Liquid Chromatography, which enabled advanced separation by the use of a C18 column, an acidic mobile phase, and gradient elusion. Also, the most phenolic acid content was measured in the ethyl acetate extract. The results revealed that all extracts were active against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Acetone extract and extract C had a potential against 4 tested bacteria. Extract A demonstrated inhibition against 3 bacteria and 3 fungi. The minimum inhibitory concentrations of the most effective extracts ranged from 11.7-187.5 µg/mL for the bacterial strains. Furthermore, methanol, B and C extracts at 0.17, 1.7, 17, 85 and 170 µg/mL concentrations were tested on rat glioma (C6) cells. Although none of the above methanol doses affected in 24 hours, 85 and 170 µg/mL slightly decreased C6 viability in 48 hours. Extract B at doses of 17, 85 and 170 µg/mL decreased C6 survival dose dependently in 24 and 48 hours. Only 170 µg/mL extract C reduced the survival ratio in 24 hours, but in 48 hours 0.17, 1.7, 17, 85 and 170 µg/mL concentrations inhibited cell viability. The present study suggests the possibility that *D. scoparium* may possess antimicrobial and anticancer molecule(s).

Key words: *Dicranum scoparium*, moss, phenolics, antifungal, antibacterial, cytotoxicity

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Dicranum scoparium' nın fenolik bileşikleri ile antimikrobiyal ve sitotoksik etkilerinin taranması

Özet

Bryofitler, ciğer otları, boynuz otları ve yapraklı kara yosunları olmak üzere karada yaşayan en eski bitki türlerindendir. Son zamanlarda yapılan araştırmalar kara yosunlarının sıra dışı ve eşsiz maddeler içerdiğini ve yüksek oranda biyolojik aktivitede bulunduklarını ortaya koymuştur. Bu araştırma, Eskişehir Sündiken Dağlarından toplanan *Dicranum scoparium* (Hedw.)'un fenolik bileşenleri ile antimikrobiyal ve sitotoksik özelliklerini ortaya koyma amacıyla yapılmıştır. Ekstraktların toplam fenol içeriği Folin-Ciocalteu' nun yaptığı çalışma temel alınarak belirlenmiş ve yapılan analizler sonucunda ekstraktların gallik asit eşdeğerleri 81,90±0,10 ile 105,21±0,10 mg/g arasında bulunmuştur. En yüksek toplam fenolik asit içeriği etil asetat ekstraktından (ekstrakt C) elde edilmiştir. Farklı polaritelere sahip çözücüler kullanılarak yapılan fenolik asit toplama işlemi, asidik mobil faz olan C18 kolonunun kullanımı ve dereceli elüsyon ile ayrım yapılmasını mümkün kılan ters fazlı Yüksek Performanslı Sıvı Kromatografisi ile yürütülmüştür. Bu işlem sonrasında yine en yüksek fenolik asit içeriği etil asetat ekstraktından elde edilmiştir.

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Antimikrobiyal analiz sonuçlarına göre tüm ekstraktların *Pseudomonas aeruginosa* ile *Bacillus subtilis*' e karşı aktif olduğu belirlenmiştir. Ayrıca, aseton ekstratı ile ekstrakt C' nin test edilen 4 bakteriye karşı etkisinin olduğu gözlenmiştir. Ekstrakt A, 3 bakteri ve 3 mantar türüne karşı inhibisyon özelliği sergilemiştir. En etkili ekstraktların minimum inhibitör konsantrasyonları bakteriler için 11,7-187,5 µg/mL olarak ölçülmüştür. Ayrıca sıçan glioma hücreleri (C6) üzerinde 0,17, 1,7, 17, 85 ve 170 µg/mL'lik konsantrasyonlara sahip metanol, B ve C ekstraktları test edilmiştir. Yirmi dört saat süre ile denenen metanol ekstraktının hücrelerde herhangi bir sitotoksik etki göstermemesine rağmen, 85 ve 170 µg/mL' lik konsantrasyonların 48 saat de C6 canlılığını hafif derecede azalttığı gözlenmiştir. 17, 85 ve 170 µg/mL'lik B ekstraktları ise, glioma hücre canlılığını doz ve zamana bağlı olarak azaltmıştır. Ekstrakt C ise 24 saatte yalnızca 170 µg/mL'lik dozunda hücre canlılığı üzerinde etki gösterirken, 48 saat saatte denenen tüm dozlarda sitotoksik etki göstermiştir. Yapılan bu çalışma *D. scoparium*' un antimikrobiyal ve antikanser moleküllere sahip olabileceğini göstermiştir.

Anahtar kelimeler: *Dicranum scoparium*, karayosunu, fenolikler, antifungal, antibakteriyal, sitotoksisite

1. Introduction

Bryophytes belong to the simplest green land plants. At the same time, they belong to the second largest taxonomic group in the plant kingdom. There are around 25,000 species of Bryophytes, which can be found in most ecosystems worldwide and include mosses (8000 species), liverworts (6000 species) and hornworts (1000 species) (Klavina et al., 2015). The phytochemistry of mosses has been neglected for a long time because they are morphologically very little and hard to gather in great amount as pure specimens; their identification is also very hard even under the microscope (Asakawa et al., 2013).

Many compounds that have been isolated from mosses have shown high biological activity. Therefore, extracts of bryophytes are prospects for the search of new pharmaceutically-active compounds (Klavina et al., 2015). Well-expressed antimicrobial and antiviral activities have been demonstrated in a number of mosses and their cytotoxic effects, antitumor, antioxidant, cardiotonic, antithrombin, insecticidal, molluscicidal and neuroprotective activity have been approved in some studies (Asakawa et al., 1980, 1985; Van Hoof et al., 1981; Ilhan et al., 2006; Sabovljevic et al., 2006; Singh et al., 2006; Jovkovic et al., 2008; Bodade et al., 2008; Veljic et al., 2008; Dülger et al., 2009; Sabovljevic et al., 2010; Elibol et al., 2011; Ertürk et al., 2015; Klavina et al., 2015). Furthermore, our previous studies demonstrated that *Homalothecium sericeum* (Hedw.) Schimp. and *Fontinalis antipyretica* (Hedw.) which belong to the bryophytes, have both antimicrobial and antiproliferative activities on rat glioma (C6) cell line (Oztopcu-Vatan et al., 2011, 2012; Savaroglu et al., 2011a).

The objective of this research was to analyze the total phenolic contents and compositions of phenolic acids, the antimicrobial and cytotoxic effects of *Dicranum scoparium* (Hedw.) (Dicranaceae) extracts and to contribute to the future studies in pharmaceutical botany.

2. Materials and methods

Plant materials were collected from Sundiken Mountains (Arikaya, Eskisehir), at an altitude of 1180 m, on soil, in July 2006. The specimen was identified in the Department of Plant Biology at Eskisehir Osmangazi University. A voucher specimen (Savaroglu 469) was deposited at the Herbarium of the Department.

2.1. Procedure of Extraction

It is only used green and brown shoots for the experiment. The plant material was washed under tap water in order to be cleaned from dead materials and stuck on debris. 0.8% density of Tween 80 aqueous solution treated to fresh gametophytic samples of *D. scoparium* in order to clean epiphytic hosts usually existing on the surface, and the samples were thoroughly washed under distilled and tap water, then left to dry on paper filter at room temperature. The materials were grained after drying through a hammer mill.

Two different processes were applied for the extraction (Tsao and Deng, 2004; Jones and Kinghorn, 2005; Ozturk et al., 2009; Oztopcu-Vatan et al., 2011; Savaroglu et al., 2011a). Yields obtained from extractions were stocked at +4°C for future use. Before using, they were weighed and then melted in dimethyl sulphoxide (DMSO) to a ultimate concentration of 200 mg/mL.

1.2. Determination of total phenolic contents

Total phenolic contents of the extracts were assigned by using Folin–Ciocalteu reagent with regard to the method of Singleton and Rossi (1965). Briefly, 100 µL of crude extract (1 mg/mL) and gallic acid (GA) standard solutions (0.02-0.18 mg/mL at concentrations) mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent for 3 minutes, followed by the addition of 1.5 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 120

minutes in the dark, and absorbance was measured at 750 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent (GAE) per g dry weight.

1.3. Analysis of phenolic acids by HPLC

An HPLC system containing of the model of 600 E HPLC pump, 717 plus autosampler, 996 photodiode array detector (PAD), and data processor (Millennium 32) was used (Waters Corp., Massachusetts, USA). Ultrapure water (18.2 µS cm-1) from a Millipore (Molsheim, France) water purification system and an octadecylsilane (ODS, C18) ultrasphere column from Teknokroma (Barcelona, Spain) (100x4.6 mm inner diameter, particle size of 3 µM) were utilized in the HPLC analysis. Ultra-pure deionized water was purified by a Millipore Synergy Water Purification System (Rotterdam, Netherland) to a specific resistance of 18 m Ω cm. Chromatographic analysis of the extracts was implemented by a gradient elution (solution A, methanol:water:formic acid [10:88:2, *v/v/v*]; solution B, methanol:water:formic acid [90:8:2, *v/v/v*]) as declared otherwhere (Öztürk et al., 2007). The analyses were carried out through a linear gradient program. Beginning condition was 100% A; 0-15 minutes, changed to 100% A; 15-20 minutes, to 85% A; 20-30 minutes, to 50%; 30-35 minutes to 0% A; 36-42 minutes, went back to 100% A. The flow-rate was 1 mL/minute, and the volume of injection volume was 10 µL. Signals were identified at 280 nm. The internal standard technique was applied to increase the repeatability. The appropriate extracts were dissolved in a mixture of methanol and water (1:1 vol/vol), and the mixture was injected into the HPLC apparatus. All the data were the average of triplicate analyses. Mean values, standard deviations (SD), medians, and both minimum and maximum contents of all the obtained results have also been determined. The correlation analysis of phenolic contents was performed through the correlation and regression program in the Microsoft EXCEL program.

2.4. Tests for Antimicrobial activity

The numbers of strain and sources of the obtained microorganisms (bacterial and fungal species) are presented in Table 1. This experiment was implemented pursuant to the method defined by the National Committee for Clinical Laboratory Standards (NCCLS, 2008), with some alterations. The test-cultures of bacteria were incubated in Mueller-Hinton Broth (MHB) at 35 to 37 ºC until they became visibly turbid. Alternatively, in order to stimulate spore formation, the molds were grown on Potato Dextrose Agar (PDA) slants at 27 ºC for 5 to 7 days. Inoculum suspensions containing 10^6 CFU/mL were used for experiments. The antimicrobial assays were carried out by modified disc-diffusion method and microdilution method (Ilhan et al., 2006; NCCLS, 2008; Oztopcu-Vatan et al., 2011; Savaroglu et al., 2011a, 2011b).

Table 1. Bacterial and fungal strains

Bacterial strains	Fungal strains
[a]*Bacillus subtilis* NRRL B-209	[c]*Aspergillus flavus* ATCC 9807
[b]*Enterococcus faecalis* ATCC 29212	[a]*Aspergillus fumigatus* NRRL 163
[b]*Escherichia coli* ATCC 25922	[c]*Aspergillus niger* ATCC 10949
[b]*Pseudomonas aeruginosa* ATCC 27853	[a]*Aspergillus parasiticus* NRRL 465
[c]*Salmonella typhimurium* ATCC 14028	[d]*Fusarium graminearum* (wild type)
[b]*Staphylococcus aureus* ATCC 25923	[d]*Fusarium solani* (wild type)
	[d]*Geotrichum candidum* (wild type)

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2.5. Cytotoxic activity

The C6 cells were cultured as described previously (Oztopcu-Vatan et al., 2011, 2012). At the beginning of the experiment the C6 cells were seeded into $2x10^4$ cells/well in 96 well plates for 24 hours incubation period. Methanol, B and C extracts were dissolved in DMSO, then diluted further in DMEM at a ratio of 1:10. The content of DMSO in the final concentrations did not go beyond 0.1%. At these concentrations, DMSO was found to be nontoxic for the tested cells. After this incubation period, the medium was replaced with only medium (control) or medium with extracts at

concentrations of 0.17, 1.7, 17, 85 or 170 µg/mL for 24 or 48 hours. Drug cytotoxicity screening was evaluated by using MTT colorimetric assay (Savaroglu et al., 2011a). The absorbance of formazan dye was read at 550 nm via a microplate reader (Bio-Tek Instruments, USA). All statistical analyses were carried out by one-way analysis of variance (ANOVA) and followed up by Tukey's multiple comparison tests. A p value less than 0.05 was considered as significant..

3. Results

Table 2 shows a summary of results of extracts obtained from *D. scoparium* employed in this study containing the extraction yields, total phenols contents for the extracts recovered with different polarity solvents. The extraction yield as a percentage of plant material ranges from 0.98% for ethyl acetate extract to 3.28% for methanol extracts. Methanol extract demonstrated a higher yield than ethyl acetate extract. The reason of this might be the presence of more polar compounds in the methanol extracts of plants.

3.1. Total phenolic contents

Total phenolic contents of extracts were spectrophotometrically determined by Folin-Ciocalteu assay. Among these tested extracts, the ethyl acetate extract had significantly higher total phenolic content compared with other extracts (as 105.21±0.10 mg GAE, g^{-1} extract) (Table 2).

1.4. Determination of phenolic acids

The chromatograms of phenolic acids of the extracts are given in Figure 1, achieved under the conditions as indicated above (Öztürk et al., 2007). In the studied extracts, nine phenolic acids (protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, o-coumaric and tr-cinnamic acids) were determined by an HPLC gradient system. All the phenolic acids were resolved completely from each other. Based on a comparing of the retention time (Rt) with standard substances, the existence of the mentioned phenolic acids was confirmed. The integrated peak areas and their retention times were computed to obtain the rate of peak normalization of the relevant phenolic acids, and their amounts were calculated in the related extracts via their calibration curves. The results showed that protocatechuic acid was the principle phenolic acid in the studied extracts and the concentration varied from one to another in the methanol and ethyl acetate extract. In addition, the other phenolic acids detected from extracts were o-Coumaric Acid, p-hydroxybenzoic acid and tr-Cinnamic Acids (Table 2).

Table 2. The extraction yields (%), total phenolic and phenolic acid contents of *D. scoparium* in different extracts

Plant Material	Extracts	Yield (%)	Phenolic acid contents (mg/100g)									)
			proCA	p-OHBA	VA	CA	SA	p-COU	FA	o-COU	tr-CIN	Total Phenol Contents (mg/g)
D. scoparium	Methanol	2.10	3.75	3.87	-	-	-	-	-	4.06	3.79	81.90± 0.10
	Ethyl Acetate	0.95	26.96	12.30	1.20	-	-	1.91	-	13.33	3.31	105.21± 0.10

proCA: protocatechuic Acid; p-OHBA: p-hydroxybenzoic Acids; VA: Vanillic Acid; CA: Caffeic Acid; SA: Syringic Acid; p-COU: p-coumaric acid; FA: Ferulic Acid; o-COU: o-Coumaric Acid; tr-CIN: tr-Cinnamic Acid

1.5. Antimicrobial activity

Bacterial and fungal strains used for antimicrobial activity test were illustrated in Table 1. The antimicrobial activities of all extracts of *D. scoparium* against bacteria and fungi were examined in the current study and their potency was qualitatively evaluated by the presence or absence of inhibition zones and zone diameter (Table 3). All extracts demonstrated an inhibitory effect against *P. aeruginosa*. Methanol extract exhibited high antimicrobial activity against *P. aeruginosa* (14 mm) and *B. subtilis* (11 mm). Extract C has the high effect against *S. aureus* (14 mm), *B. subtilis* (13 mm), *P. aeruginosa* (12 mm) and *E. faecalis* (11 mm). The inhibition zone produced by extract B against *P. aeruginosa* (13 mm), *B. subtilis* (11 mm) and *S. aureus* (8 mm). However, aqueous extract D was found to be inactive except *P. aeruginosa* and *B. subtilis* (Table 3). The applied concentration of all extracts of *D. scoparium* did not demonstrate any activity against *E. coli* and *S. typhimurium*. Upon comparing all the extract, methanol extract and extract C exhibited the highest antimicrobial activity against *P. aeruginosa* and *S. aureus*, respectively. Extract A and Chloroform extract exhibited some degree (9 mm) of activity against *A. fumigatus*, *F. solani*, *G. candidum*. Table 4 illustrates the MIC ranges of extracts methanol, extract B and C against bacterial strains. The MIC of the extracts ranged from 11.70-187.50 µg/mL.

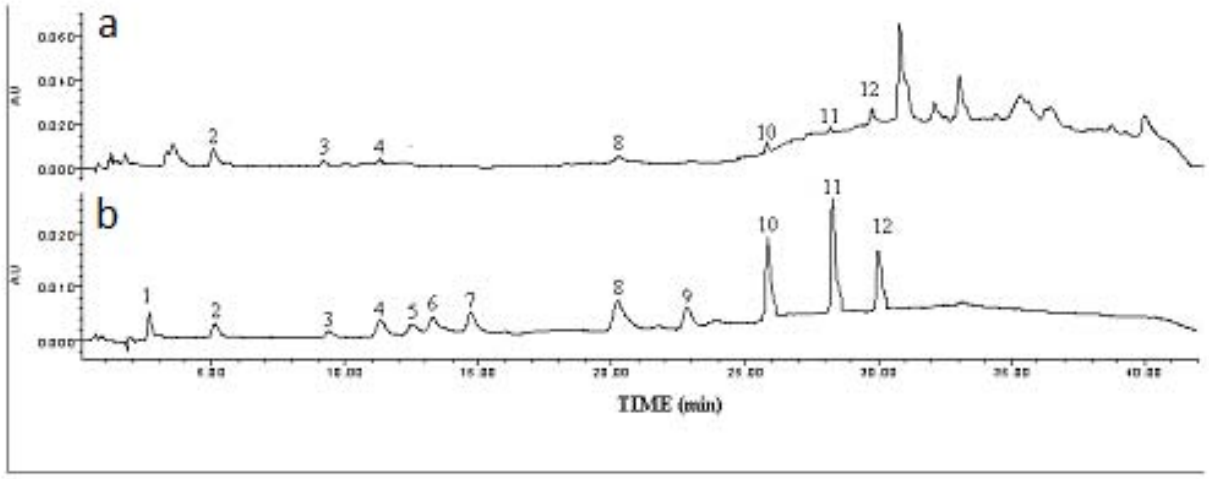

Figure 1. The representative chromatogram of ethyl acetate extracts of *D. scoparium* (a) and the mixture of standard phenolic acids and IS (b). The phenolic acids are symbolized by GA (1), protoCA (2), *p*-hydBA (3), VA (4), CA (5), ChA (6), SA (7), *o*-COU (8), FA (9), *p*-COU (10), tr-CIN (11) and IS (propyl paraben (12) on the chromatograms.

Table 3. Antibacterial and antifungal activities of *D. scoparium* extracts as inhibition zones (mm).

Bacterial Strains	Methanol	Chloroform	Acetone	Extract A	Extract B	Extract C	Extract D	Control 1*	ontrol 2**	C
B. subtilis 209	11	10	10	10	11	13	8	13±0.2	4±0.1	2
E. coli 25922	NS	NS	NS	NS	NS	NS	NS	30±0.2	5±0.1	2
E. faecalis 29212	NS	10	8	NS	NS	11	NS	27±0.2	5±0.1	1
P. aeruginosa 27853	14	12	10	12	13	12	10	30±0.2	0±0.1	2
S. typhimurium 14028	NS	NS	NS	NS	NS	NS	NS	21±0.2	8±0.1	1
S. aureus 25923	NS	NS	11	8	8	14	NS	35±0.2	7±0.1	2
Fungal Strains									Control 3***	
A. flavus 9807	NS	NS	NS	NS	NS	NS	NS	7±0.1		
A. fumigatus 163	NS	NS	NS	9	NS	NS	NS	15±0.1		
A. niger 10949	NS	NS	NS	NS	NS	NS	NS	13±0.1		
A. parasiticus 465	NS	NS	NS	NS	NS	NS	NS	14±0.1		
F. graminearum	NS	NS	NS	NS	NS	NS	NS	16±0.1		
F. solani	NS	NS	NS	9	NS	NS	NS	13±0.1		
G. candidum	NS	9	NS	9	NS	NS	NS	11±0.1		

NS: not sensitive; * Penicillin (10 µg/disc); ** Tetracycline (30 µg/disc); *** Amphotericin B (10 µg/disc)

Table 4. MIC of Extract Me, B and C (µg/mL)

Bacterial strains	Me	B	C	Penicilin (µg/ml)	Tetracycline (µg/ml)
B. subtilis	-	-	93.75	<1.5	<1.5
S. aureus	-	-	187.50	<1.5	<1.5
P. aeruginosa	11.70	23.40	-	<1.5	<1.5

3.4. Cytotoxic activity

Although none of methanol doses affected for 24 hours, in 48 hours only 85 and 170 µg/mL doses had a slight decreasing effect on cell survival about 18 ($p<0.01$), 38% ($p<0.001$), respectively (Figure 2, 3). Cell viability was reduced by 16 ($p<0.01$), 29 ($p<0.001$) and 33% ($p<0.001$) at 17, 85 and 170 µg/mL concentrations of extract B for 24 hours (Figure 2). The highest concentration of extract B had strong cytotoxic activity on glioma cells and IC_{50} value of extract B was calculated as 50 µM for 48 hours (Figure 3). After 24 hours incubation, while the cell survival rates did not change at 0.17, 1.7, 17 and 85 µg/mL of extract C, 170 µg/mL reduced by 44% ($p<0.001$) (Figure 2). However, in

48 hours 1,7, 17, 85 and 170 µg/mL extract C were able to reduce cell survival about 13, 50, 70 and 84% ($p<0.001$), respectively. IC_{50} value of extract C was calculated as 50 µM in 48 hours (Figure 3).

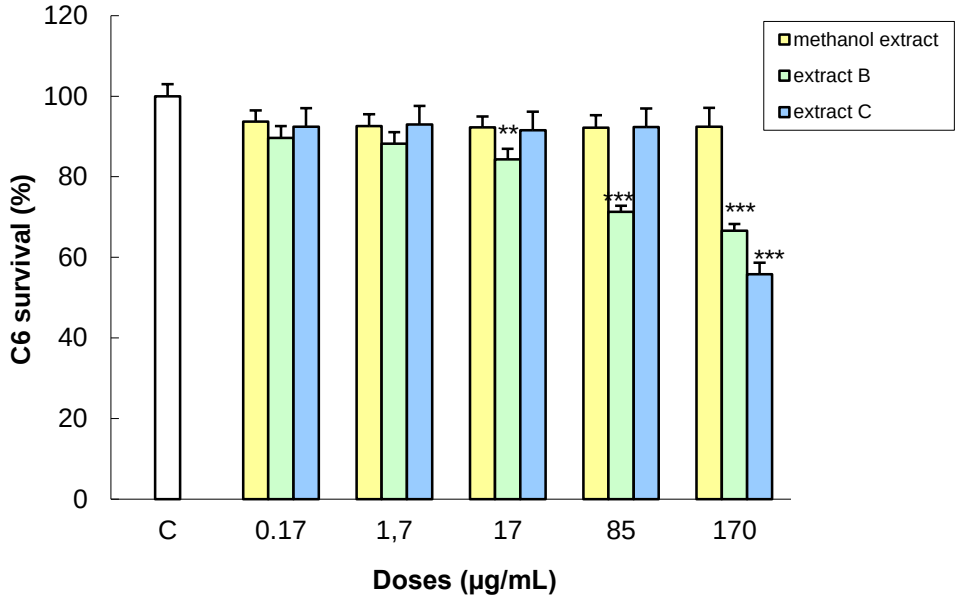

Figure 2. The effect of methanol extract, extract B and C of *D. scoparium* on C6 cell survival for 24 hours (C: Control, **: $p<0.01$, ***: $p<0.001$)

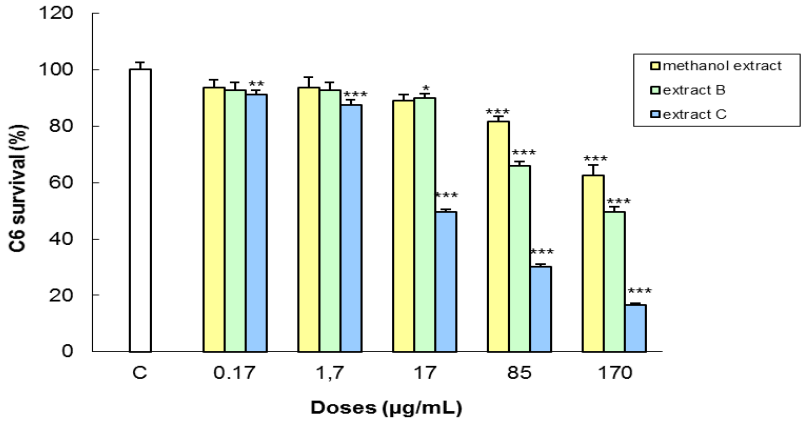

Figure 3. The effect of methanol extract, extract B and C of *D. scoparium* on C6 cell survival for 48 hours (C: Control, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$)

4. Conclusions and discussion

Bryophytes are attractive group in plant botany. Mosses have high biologic activities at significant levels. Therefore, we studied for the first time the possible antimicrobial, cytotoxic activities and the total phenolic contents and compositions of phenolic acids of some extracts of *D. scoparium*.

The chemical composition of bryophytes and that there is small amount of the same species available for analysis usually by sophisticated methods (Jockovıc et al., 2008) causes the difficulty in identification. Until now, many different secondary metabolites from Bryophytes have been obtained, such as terpenoids (mono-, di-, tri-), phenolics (flavonoids, xanthones and benzoic and cinnamic derivatives), lignins, lipids, fatty acids, steroids, carotenoids and some aromatic compounds, etc. (Sabovljevic et al., 2001; Asakawa, 2007). In addition, the presence of these phenolic acids is reported for the first time in *D. scoparium*. In the methanol extract *D. scoparium* was characterized by the presence of four phenolic acids (protocatechuic, p-hydroxybenzoic, o-coumaric, tr-cinnamic acids). Protocatechuic, p-hydroxybenzoic, vanillic, p-coumaric, o-coumaric, tr-cinnamic acids were also found in the ethyl acetate extract. In mosses, caffeic, p-coumaric and ferulic acids are known to exist (Jockovıc et al., 2008). In addition, it is recognized that phenolic compounds responsible for cytotoxic and antimicrobial properties.

The antimicrobial test results demonstrated that *D. scoparium* extracts had a potential activity against *B. subtilis, P. aeruginosa, S. aureus* and *E. faecalis*. It is known conventional antibiotics are usually more active against the gram positive bacteria than gram negative bacteria (Veljic et al., 2008). However, acrocarpic mosses showed inhibition effect against both the gram positive and gram negative bacteria (Elibol et al., 2011). Some researchers found out antimicrobial activities of different bryophyte samples against gram negative bacteria (Ilhan et al., 2006; Bodade et al., 2008; Oztopcu Vatan et al., 2011; Ertürk et al., 2015). Similar work carried out by Klavina et al. (2015) showed that extracts of *Dicranum polysetum* antibacterial activity was found *B. cereus*.

The methanol extract demonstrated activity in a high zone (14 mm) of inhibition against *P. aeruginosa*. *P. aeruginosa* is frequently associated with infections of the urinary and respiratory tract in humans. Ertürk et al. (2015) studied about antifungal and antibacterial activities of *Ctenidium molluscum* and reported crude ethanol extract was the highest antimicrobial effect with 19 mm/15 µL inhibition zone against only strain *P. aeruginosa*.

The MIC of the extracts ranged from 11.70-187.50 µg/mL. The MIC value of extract C was 187.5 µg/mL against *S. aureus.* Veljic et al. (2008) studied antifungal and antibacterial activities of methanol extracts of 8 different mosses and reported that the extracts of *D. scoparium* showed activity with a MIC of 25.0 mg/mL against *S. aureus*. In some last studies, many evaluations of moss extracts showed various levels of inhibition activity against some tested bacteria and fungi. Ertürk et al. (2015) reported that crude extract obtained from *H. cupressiforme* for *Klebsiella pneumoniae*, *B. cereus* and *Saccharomyces cerevisiae* was ≥12.5 mg/mL for *S. aureus* was ≥ 50 mg/mL. Singh et al. (2006) reported that some of the extracts like the ethanolic extract of *Plagiochasma appendiculatum* gave very low MIC values, and this species inhibited the growth of *E. coli, Proteus mirabilis, S. typhimurium* and *Trichophyton rubrum* with concentration of 2.5 µg/disc.

Extract A and chloroform extract showed some degree of activity against *A. fumigatus*, *F. solani*, *G. candidum* respectively. Ertürk et al. (2015) studied the antimicrobial activity of ethanol extracts of 8 different mosses and indicated that all the tested mosses showed a moderate antifungal activity on *A. niger, Candida albicans* and *Sacchromyces cerevisiae*. Veljic et al. (2008) reported that the extract of *D. scoparium* possessed the high antifungal activity a MIC of 0.5-2.5 mg/mL.

The cytotoxic activity results showed that only two higher concentrations of methanol extract have a partial effect on C6 growth for 48 hours. Furthermore, high doses of extract B possess a dose and time dependent cytotoxic activity on glioma cells in two time duration. Although lower doses did not affect, the cytotoxicity was prominent and time, dose-dependent with higher doses of extract C on C6 growth for 48 hours.

Similarly, our previous researches showed that extract C of *H. sericeum* and *F. antipyretica* have strong growth inhibitory effect on C6 cells (Oztopcu-Vatan et al., 2011; Savaroglu et al., 2011a). There are studies supporting our data. Extracts of some bryophytes from various species showed cytotoxic effects on various cancer cell lines (Fu et al., 2009; Krzaczkowski et al., 2009). Yamada et al. (2007) indicated that the cytotoxicity of Canadian *Sphagnum* peat on rat basophilic leukemia by MTT assay was studied and found that low doses did not show any decreasing effect after 48 hours. Sanionin A and B, from *Sanionia georgico-uncinata* (moss), was determined to have antiproliferative effect after 72 hours on mouse fibroblast and human cervix cells (Yamada et al., 2007; Ivanova et al., 2007). Ether extract of Indonesian and Tahitian *Frullania* species exhibited cytotoxic activity against both the HL-60 and KB cell lines. The EC_{50} values were estimated 6.7 and 1.6 µg/mL and 1.6 and 11.2 µg/mL, respectively (Komala et al., 2011).

The phenolics might be the major active component responsible for the strong pharmacological activity. However, more detailed enquiries between the individual phenolic compounds present in mosses and the pharmacological activities needs to be implemented. The tested methanol, B and C extracts do show antimicrobial activity on several bacteria strains (*B. subtilis, S. aureus, P. aeruginosa*). Our results showed that extract C is the most potent cytotoxic effect on glioma cells among the all extracts. This study pointed that *D. scoparium* extracts have biological active antimicrobial and cytotoxic molecule(s). These results clearly indicate that extracts investigated should find a practical application in the prevention and protection of bacterial infections of plant, animals and humans.

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