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SCREENING OF ANTIMICROBIAL, CYTOTOXIC EFFECTS AND PHENOLIC COMPOUNDS OF THE MOSS *AULACOMNIUM ANDROGYNUM* (HEDW.) SCHWAGR (BRYOPHYTA)

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ABSTRACT

The second largest taxonomic group in the kingdom plantae is bryophytes, however; studies carried out to better understand their chemical composition are limited and dispersed. In this study, phenolic compounds, the antimicrobial and cytotoxic activities of different extracts from *Aulacomnium androgynum* (Hedw.) Schwagr. Total phenolic contents of the extracts' were determined by Folin-Ciocalteu assay and their amount ranged from 41.79 to 98.41mg/g of gallic acid equivalents. The highest total phenolic content was obtained from ethyl acetate (C) extract. Phenolic acids in the extracts acquired by using different solvents were eluted through reversed-phase high-performance liquid chromatography, which enabled advance elution by the use of a C18 column, an acidic mobile phase, and gradient elution. Also, the most phenolic acid content was measured in the ethyl acetate extract. The antimicrobial activity of these extracts was evaluated against 6 bacterial and 7 fungal strains. Except extract D, the all extracts were active against *Pseudomonas aeruginosa,* and *Bacillus subtilis*. Acetone, petroleum ether (A) and ethyl acetate (C) extracts demonstrated inhibitory activity against *Staphylococcus aureus* and *Enterococcus faecalis*, but A and C were active against mold strains. The minimum inhibitory concentrations of the most effective extracts ranged from 93.75-375 µg/mL for the bacterial strains. The cytotoxic activity of different concentrations of methanol, A and C extracts were tested on rat glioma (C6) cells. Methanol extract decreased C6 viability in 48 hours at the concentration of 85 and 170 µg/mL. All doses of extract A stimulated C6 growth for 24 hours, but 170 µg/mL inhibited in 48 hours. IC_{50} value of extract C was calculated as 83 µM in 48 hours. The present study suggests the possibility that *A. androgynum* may possess antimicrobial and anticancer molecule(s).

Keywords: *Aulacomnium androgynum*, Moss, Phenolics, Antifungal, Antibacterial, Toxicity.

INTRODUCTION

The bryophytes (mosses, liverworts and hornworts) stand for the second largest group of the simplest green land plants following angiosperms, and are phylogenetically placed between the algae and the pteridophytes. The bryophytes are found everywhere in the world except the seas. There are about 20.000 and 25.000 species in the world (Asakawa 2007b; Asakawa *et al.*, 2013). There is a lack of knowledge about the chemistry of bryophytes. The reason of this situation is troubles met during identification processes, and small amount of the same species available for analyses, usually by sophisticated methods and inconspicuous position in the ecosystem (Zinsmeister *et al.*, 1991; Kumar *et al.*, 2000; Jovkovic *et al.*, 2008).

Since such biological compounds as aromatic and phenolic substances in bryophytes, aliphatic compounds and fatty acids are preserved against organisms such as mammals, insect larvae, bacteria, fungi, snails, and slugs, almost all species of bryophytes are not damaged by these organisms (Asakawa 2001, 2007b; Xie and Lou 2009; Elibol *et al.*, 2011). Therefore, a number of bryophytes (in particular, mosses) have widely been used as medicinal plants in China with the purpose of treating burns, bruises, external wounds, etc. (Asakawa 2007b). Bryophytes are regarded as a "remarkable reservoir" of new, natural products or subsidiary compounds, many of which have shown interesting biological activity (Sabovljevic *et al.*, 2009). These activities of bryophytes include cytotoxic, antitumor, cardiotonic, antifungal, antimicrobial, allergy causing, insect anti-feedant, insecticidal, molluscicidal, pesticidal, and plant growth regulatory features (Asakawa *et al.*, 1980; Van Hoof *et al.*, 1981; Asakawa *et al.*, 1985; Basile *et al.*, 1998a, b, c, 1999; Ilhan *et al.*, 2006; Sabovljevic *et al.*, 2006; Singh *et al.*, 2007; Jovkovic *et al.*, 2008; Bodade *et al.*, 2008; Veljic *et al.*, 2008; Dülger *et al.*, 2009; Sabovljevic *et al.*, 2010; Elibol *et al.*, 2011). 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; Savaroglu *et al.*, 2011).

The aim of this study was to analyze the total phenolic contens and compositions of phenolic acids, the antimicrobial and cytotoxic effects of *Aulacomnium androgynum* (Hedw.) Schwagr. (Aulacomniaceae) extracts and to contribute to the future studies in pharmaceutical botany.

MATERIALS AND METHODS

Plant materials were collected from Sundiken Mountains (Arikaya, Eskisehir), at an altitude of 1240 m, on tree roots, in July 2006. The specimen was identified in the Department of Plant Biology at Eskisehir Osmangazi University. A voucher specimen (Savaroglu 482) was deposited at the Herbarium of the Department.

Procedure of Extraction: It is only used green brown and/or green 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 *A .androgynum* Hedw. Schwagr. 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. For the first process, 10 g of the pulverized sample was extracted through 250 mL of 80% methanol, acetone, and chloroform for 8 hours via soxhlet apparatus. After being filtered with Whatman paper filter, all extracts were concentrated by rotary evaporation to dryness in vacuum (yield= 4.66, 2.46 and 2.43% respectively) and stored at +4°C for future use (Jones and Kinghorn 2005).

The second process of extraction was carried out in four steps. Firstly, 30 g of pulverized gametophytic plant sample was extracted with 250 mL of petroleum ether for 8 hours via soxhlet, and the solvent was cleared under reduced pressure on a rotary evaporator (extract A, yield 0.82%). At the second stage, non-fat material dried through air (15 g) was extracted four times with methanol: water (70:30, v/v) at 40°C, 30 minutes. The extract was then concentrated to dryness in vacuum (extract B, yield 3.27%). The two remaining extracts were prepared as follows: non-fat material dried in air (15 g) was extracted four times via methanol: water (70:30 v/v) at 40°C, 30 minutes, and this material was concentrated in vacuum for vaporization with methanol, and the remnant aqueous phase was extracted with ethyl acetate at 25°C. This was then concentrated to dryness in vacuum (extract C, yield 0.96%). The aqueous solution was concentrated separately by rotary evaporation to dryness in vacuum (extract D, yield 2.23%) (Tsao and Deng 2004; Jones and Kinghorn 2005; Ozturk *et al.*, 2009). These yields were stored at +4°C for further use. Before being used, they were first weighed and then dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 200 mg/mL.

Assignation 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). Concisely, 0.1 mL of Gallic acid (GA) (various concentrations) and sample solutions were diluted with 5.0 mL methanol 0.5 mL of 10% Folin-Ciocalteu reagent and 1.5 mL of 20% Na_2CO_3 was also added, and after vortexing, the mixture was left for incubation for 2 hours at room temperature. The result of the measurement of the absorbance was 750 nm at the end of the incubation period. The total phenolic content was expressed as mg GA equivalents per g of sample.

Analysis of phenolic acids by HPLC: An HPLC system containing of the model of 600 E HPLC pump, 717 plus auto sampler, 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 reported elsewhere (Öztürk *et al.*, 2007). The analyses were carried out through a linear gradient program. The initial condition was 100% A; 0-15 min, changed to 100% A; 15-20 min, to 85% A; 20-30 min, to 50%; 30-35 min to 0% A; 36-42 min, went back to 100% A. The flow-rate was 1mL/minute, and the volume of injection volume was 10 µL. Signals were identified at 280nm. The internal standard technique was applied to increase the repeatability. The relevant 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.

Microorganisms: Bacterial strains were recovered from long-term storage at –80°C in the cryobank. The bacteria were vitalized in Nutrient Broth (Merck, Germany) at 35 to 37°C, and then inoculated on Nutrient Agar (Merck) plates to be checked in terms of microbial purity. The molds were vitalized in Malt Extract Agar (Merck) at 27°C. The numbers of strain and sources of the obtained microorganisms are presented in Table 1.

Antimicrobial activity: 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. The density of these cultures was set to the same turbidity of the 0.5 McFarland standard (at 625 nm, 0.08 to 0.1 absorbance) with sterile saline. Alternatively, in order to stimulate spore formation, the molds were bred on Potato Dextrose Agar (PDA) slants at 27 ºC for 5 to 7 days. After being counted with the Thoma slide, the spore concentration for each mold was set right to 10^6 CFU/mL with sterile 0.1% Tween 80. Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates, each of which was cooled down to 45 to 50°C and sterilized, were used for bacteria and fungi, respectively, and then the plates were placed into the sterilized Petri dishes (9 cm). The whole surface of the MHA plates and the Sabouraud 4% Glucose Medium plates were inoculated with the bacteria and fungi by being spread with a sterile swab dipped into the adapted suspensions. Six wells, each of which were 6 mm in diameter, were cut out of the agar, and 20 µL of the extract solutions was placed into each well. The plates were incubated with bacteria at 37°C for 24 hours or with fungal strains at 30°C for 48 hours. After these incubations, the dishes were kept at 4°C for 2 hours. The diameters of the inhibition zones were measured in terms of millimeters. As a positive control for bacteria penicillin and tetracycline (Bioanalyses), and for fungi, amphotericin B (Sigma) was used. DMSO was used as the negative control. All assays were done in duplicate.

Minimum inhibitory concentration (MIC): Micro dilution method was utilized through a 96 well plate pursuant to NCCLS (NCCLS 2008) for the determination of MIC. Firstly, 100 µL of MHB or Sabouraud Dextrose Broth was placed in each well. The stock solutions of the extracts were diluted and transferred into the first well, and serial dilutions were performed so that concentrations in the range of 1.5 to 1500 µg/mL could be acquired. The inoculums were set right to contain approximately 10^5 CFU/mL bacteria and 10^4 CFU/mL fungi as defined above. 100 µL of the inoculums was added to all the wells and the plates were incubated at 37ºC for 24 hours for bacteria or at 30ºC for 48 hours for fungi. MIC values were determined by adding 20 µL of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution. The MIC value was considered as the lowest concentration of the extract that inhibited any visible bacterial or fungal growth, as indicated by TTC staining following the incubation (NCCLS, 2008). As the reference antibiotic control, it is used again penicillin and tetracycline.

Cytotoxic activity: The C6 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 10% heat-inactivated fetal bovine serum (Sigma), 1% 100 U/mL penicillin and 100 µg/mL streptomycin solution (Biochrom). Cells were cultivated at 37°C in a moisturized atmosphere of 95% air and 5% CO_2. The C6 cell viability was achieved by trypan blue (Sigma) dye exclusion method and seeded into $2x10^4$ cells/well in 96 well plates for 24 hours incubation period. Methanol, A 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. All extracts were prepared immediately before use and protected from light. 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. Each tested concentration was inoculated at least in eight wells and all experiments were performed at least three times. After 24 or 48 hours drug cytotoxicity screening was evaluated by using 3-(4,5-dimethylthiazol-2yl)- 2,5-diphenyltetrazolium bromide (MTT, Applichem) colorimetric assay (Oztopcu-Vatan *et al.,* 2011). The absorbance of formazan dye was read at 550 nm via a microplate reader (Bio-Tek Instruments, USA). All statistical analyses were performed using 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.

RESULTS

Table 2 shows a summary of results of extracts obtained from *A. androgynum* 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 0.96 for extract C to 4.66 for ME extracts. Methanol extract demonstrated a higher yield than others. The reason of this might be the presence of more polar compounds in the methanol extracts of plants.

Total phenolic contents: Total phenolic contents of extracts were spectrophotometrically determined by Folin-Ciocalteu assay. Among these tested extracts, the extract C (ethyl acetate extract) had significantly higher total phenolic content compared with other extracts (as 98.91 mg GA, g^{-1} extract) (Table 2).

HPLC analysis 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, ten phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, caffeic, chlorogenic, syringic, *p*-coumaric, ferulic, *tr*-cinnamic and *o*-coumaric 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 extract C. In addition, the other phenolic acids detected from extracts were *tr*-cinnamic, vanillic, *p*-hydroxybenzoic acids (Table 2).

Antimicrobial activity: Bacterial and fungal strains used for antimicrobial activity test were illustrated in Table 1. The antimicrobial activities of all extracts of *A. androgynum* 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*. Extract A exhibited high antimicrobial activity against *P. aeruginosa* (26 mm), *S. aureus* (14 mm), with modest activity against *B. subtilis* (10 mm) and *E. faecalis* (8 mm).The inhibition zone diameter against *P. aeruginosa* for extract A was bigger than those of the standard antibiotic tetracycline (30 µg/disc) (Table 3).Extract B has the modest effect against only *P. aeruginosa* (11mm) and *B. subtilis* (10 mm).The inhibition zone produced by extract C against *B. subtilis*, *P. aeruginosa*, *E. faecalis* and *S. aureus* were almost 12 mm. However, aqueous extract (D) was found to be inactive except *P. aeruginosa*. All the extracts showed no antifungal activity except extract A and C. Table 4 illustrates the MIC ranges of extracts A and C against bacterial strains. The MIC of the extracts ranged from 93.75-375 µg/mL.

Cytotoxic activity: Although all doses of methanol extract in 24 hours did not change, in 48 hours 85 and 170 µg/mL decreased C6 viability about 14 ($p<0.01$), 47% ($p<0.001$), respectively. C6 cell viability was stimulated by 17, 16, 23 and 41% at 1.7, 17 ($p<0.05$), 85 ($p<0.01$) and 170 ($p<0.001$) µg/mL concentrations of extract A for 24 hours (Figure 2). On the contrary, all doses of extract A were found to be inactive on cell viability except 170 µg/mL. The highest concentration of extract A inhibited C6 viability by 24% ($p<0.001$) in 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 48% ($p<0.001$). However, in 48 hours 85 and 170 µg/mL extract C were able to reduce cell survival about 53% and 83% ($p<0.001$), respectively. IC_{50} value of extract C was calculated as 83 µM in 48 hours.

Table 1. Bacterial and fungal strains used for antimicrobial activity test.

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

Plant Material	Extracts	Yield (%)	Phenolic acid contents (mg/100g)								Total Phenol Contents (mg/g)
			proCA	p-OHBA	VA	CA	SA	FA	o-COU	tr-CIN	
A. androgynum	Methanol	4.66	6.08	1.31	1.75	-	-	-	-	3.83	41.79± 0.10
	Extract C	0.96	26.89	6.78	2.44	0.21	2.43	1.33	0.96	0.65	98.91± 0.10

proCA: Protocatechuic Acid; p-OHBA: *p*-hydroxybenzoic Acid; VA: Vanillic Acid; CA: Caffeic Acid; SA: Syringic Acid; FA: Ferulic Acid; o-COU: *o*-Coumaric Acid; tr-CIN: *tr*-Cinnamic Acid

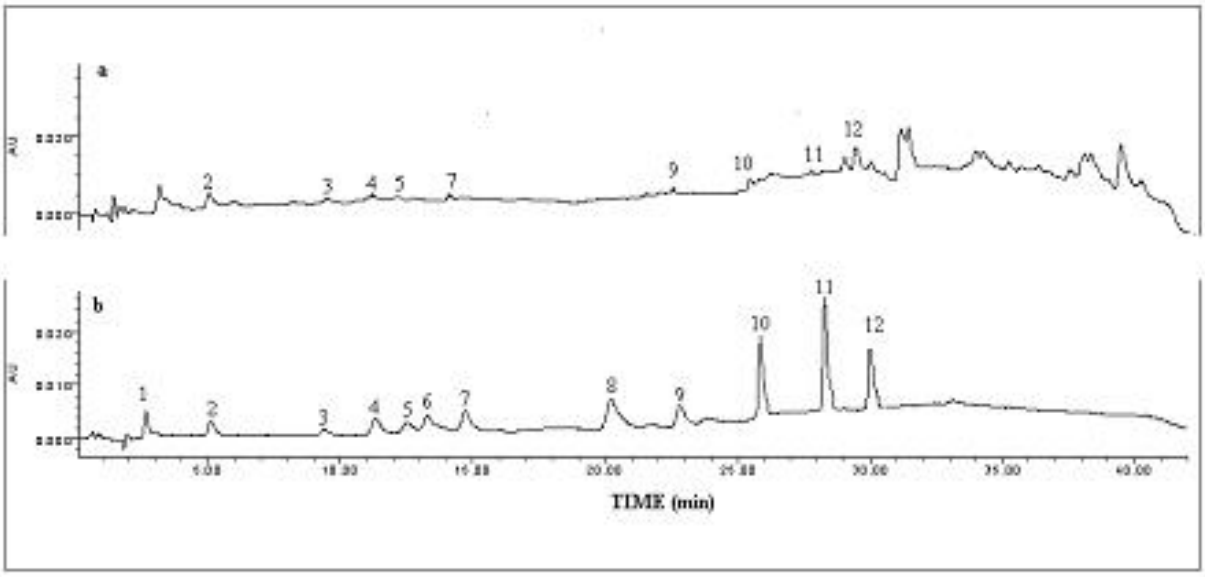

Figure 1. The representative chromatograms of ethyl acetate extracts of *A. androgynum* (a) and the mixture of standart phenolic acids and IS (b). The phenolic acids are symbolized by GA (1), protoCA (2), *p*-hydBA (3), VA (4), CA (5), ChA (6), SA (7), *o*-COU (8), FA (9), *p*-COU (10), tr-CIN (11) and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

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

BACTERIAL STRAINS	Methanol	Chloroform	Acetone	Extract A	Extract B	Extract C	Extract D	Control 1*	Control 2**
B. subtilis 209	10	10	10	10	10	12	NS	13±0,2	24±0,1
E. coli 25922	NS	NS	NS	NS	NS	NS	NS	30±0,2	25±0,1
*E. faecalis*29212	NS	9	9	8	NS	12	NS	27±0,2	15±0,1
P. aeruginosa 27853	10	10	11	26	11	12	8	30±0,2	20±0,1
S. typhimurium 14028	NS	NS	NS	NS	NS	NS	NS	21±0,2	18±0,1
S. aureus 25923	NS	NS	9	14	NS	12	NS	35±0,2	27±0,1
FUNGAL STRAINS								Control 3***	
A. flavus 9807	NS	NS	NS	NS	NS	NS	NS	7±0,1	
A. fumigatus 163	NS	NS	NS	NS	NS	8	NS	15±0,1	
A. niger 10949	NS	NS	NS	NS	NS	NS	NS	13±0,1	
A. parasiticus 465	NS	NS	NS	9	NS	NS	NS	14±0,1	
F. graminearum	NS	NS	NS	NS	NS	NS	NS	16±0,1	
F. solani	NS	NS	NS	NS	NS	NS	NS	13±0,1	
G. candidum	NS	NS	NS	NS	NS	NS	NS	11±0,1	

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

Table 4. Minimum inhibitory concentrations (MIC, µg/mL) of Extract A and C.

Bacterial strains	A	C	Penicillin(µg/ml)	Tetracycline(µg/ml)
B. subtilis	-	187,50	<1,5	<1,5
E. faecalis	-	187,50	-	-
P. aeruginosa	187,50	93,75	<1,5	<1,5
S. aureus	375,00	187,50	<1,5	<1,5

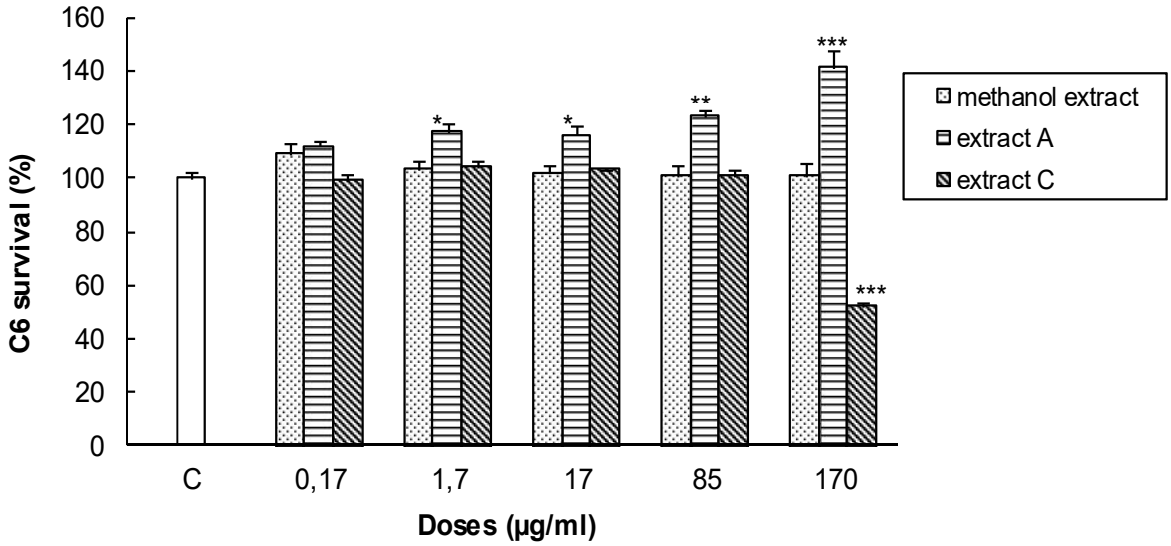

Figure 2.The effect of methanol extract, extract B and C of *Aulacomnium androgynum* on C6 cell survival for 24 hours (C: control, *: p<0.05, **: p<0.01, ***: p<0.001).

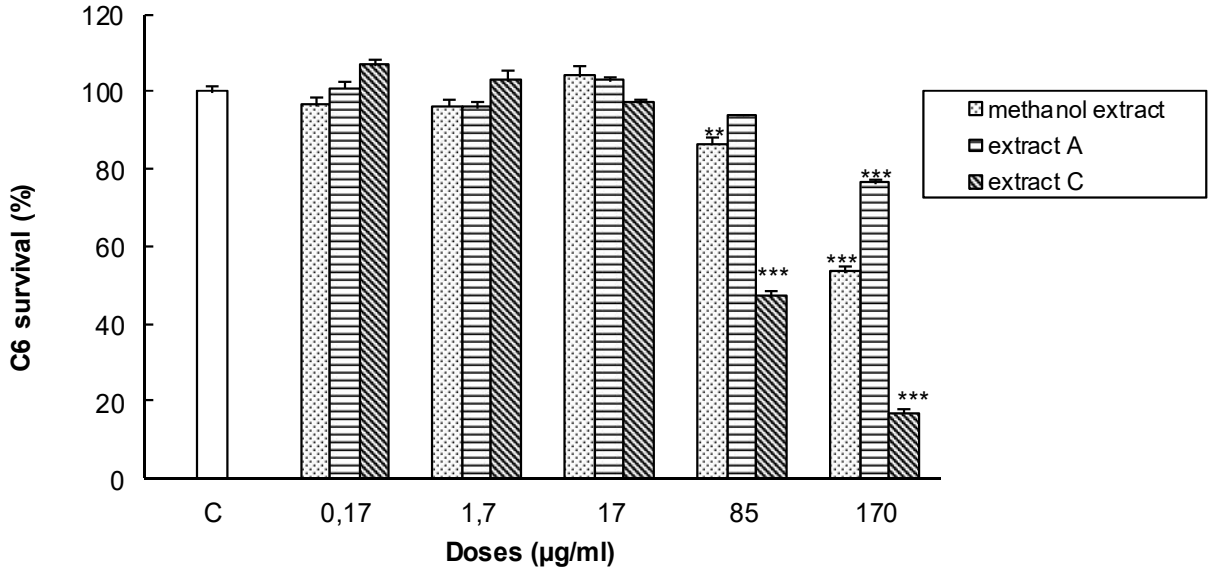

Figure 3.The effect of methanol extract, extract B and C of *Aulacomnium androgynum* on C6 cell survival for 48 hours (C: control, **: p<0.01, ***: p<0.001).

DISCUSSION

Bryophytes are very interesting group in botany and few studies have been realized in chemistry, especially on molecular level. 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 *A. androgynum*.

The chemical composition of bryophytes and that there is small amount of the same species available for analysis usually by sophisticated methods (Jockovic *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 2007a). In addition, the presence of these phenolic acids is reported for the first time in *A. androgynum*. In the methanol extract *A. androgynum* was characterized by the presence of four phenolic acids (protocatechuic, *p*-hydroxybenzoic, vanillic, *tr*-cinnamic acids). Protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, ferulic, *o*-coumaric, *tr*-cinnamic acids were also

found in the extract C. In mosses, caffeic, *p*-coumaric and ferulic acids are known to exist (Jockovic *et al.*, 2008). In addition, it is recognized that phenolic compounds carry cytotoxic and antimicrobial properties.

The antimicrobial test results demonstrated that *A. androgynum* extracts had a potential activity against *B. subtilis, S. aureus, E. faecalis* and especially *P. aeruginosa*. It is known conventional antibiotics are usually more active against the gram positive bacteria than gram negative bacteria. However, the bryophyte extracts demonstrated inhibitory effect against both the gram positive and negative bacteria. Similarly, some researchers found out antimicrobial activities of different bryophyte samples against gram negative bacteria (Basile *et al.*, 1998b, c; Ilhan *et al.*, 2006; Elibol *et al.*, 2011; Oztopcu-Vatan *et al.*, 2011). In a study researching the biological activity of 13 types of mosses, Klavina *et al.*, (2015) reported that all the extracts have an effect upon both gram positive and gram negative bacteria.

In the last 30 years, we have noticed an increase in resistance to antimicrobial agents, leading to the use of antibiotics repeatedly and insufficiency in disease control. Owing to the rising prevalency of antibiotic-resistant pathogens in hospitals and at homes, a deliberate search is in progress for alternative treatments in order to more effectively fight against further spread of antibiotic-resistant pathogens (Baravalia *et al.*, 2009). The extract A demonstrated activity in a large zone (26 mm) of inhibition against *P. aeruginosa*. *P. aeruginosa* is frequently associated with infections of the urinary and respiratory tract in humans. It is a striking fact that extract A is highly effective against pathogen microorganism and it may be used as possible natural antimicrobial agents to control various human and animal diseases.

The MIC of the extracts ranged from 93.75-375 µg/mL. The MIC values of extract A and C were 187.5 and 93.75µg/mL, against *P. aeruginosa* respectively. Similar work carried out by Oztopcu-Vatan *et al.*, (2011) showed that acetone, extract A and C of *Homalothecium sericeum* have MIC of 23.4-375 µg/mL for *P. aeruginosa*. 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 C showed less antifungal activity against *A. parasiticus* and *A. fumigatus* respectively. In the previous studies, no antifungal activity was recorded in selected bryophytes (Basile *et al.*, 1998a; Ilhan *et al.*, 2006; Oztopcu-Vatan *et al.*, 2011). On the other hand, Veljic *et al.*, (2008) studied the antimicrobial activity of methanol extracts of different mosses and indicated that all the tested extracts showed a strong antifungal activity. At the same time, Mewari and Kumar (2008) also found that *Marchantia polymorpha* (methanol extract) was active on *Candida albicans* and *T. mentagrophytes*. Ertürk *et al.*, (2015) studied antifungal and antibacterial activities of 8 different acrocarpous mosses and reported that the extracts of *H. sericeum* species showed the highest antibacterial and antifungal activity against *Y. enterocolitica*, *S. typhimurium* and *S. cerevisiae* (16.33, 16, and 25 mm/15 µL inhibition zones, respectively).

The cytotoxic activity results showed that only two higher concentrations of methanol extract have a partial effect on C6 growth for 48 hours. Interestingly, among the extracts, only extract A stimulated cell growth dose-dependent manner for 24 hours. The 170 µg/mL caused the cell proliferation by 41%, however, we did not observe this effect after 48 hours treatment. The highest concentration of extract A was seen as less toxic on cell viability by 24% in 48 hours. We found that at high doses extract C possess a dose and time dependent anticancer activity against glioma cells. The highest concentration of extract C has anti-proliferative potential for 24 hours. 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. Furthermore, our previous studies showed that extract C of *H. sericeum* and *F. antipyretica* have strong cytotoxic activity on C6 cells (Oztopcu-Vatan *et al.*, 2011; Savaroglu *et al.*, 2011). In support of our data, Fu *et al.*, (2009) and Krzaczkowski *et al.*, (2009) determined that some bryophytes extracts from different species showed cytotoxic effects on various human cancer cell lines. In addition, 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 the moss *Sanioniageorgico-uncinata*, was determined to have antiproliferative action after 72 hours on human leukemia cells, mouse fibroblast cells, and human cervix carcinoma 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 (HL-60 cells) and 1.6 and 11.2 µg/mL (KB cells), respectively (Komala *et al.*, 2011).

Conclusion: The results of our study clearly indicate that *A. androgynum* extracts might possess a novel antimicrobial and cytotoxic molecule(s). 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 obtained results show that mosses may be used as possible natural antioxidant, antimicrobial agents to control various human, animal and plant diseases.

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