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Original article

In vitro medicinal potentials of *Bryum capillare*, a moss sample, from Turkey

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ABSTRACT

In this study was conducted the in vitro antimicrobial, antibiofilm, antioxidant, antigenotoxic and anticancer activities investigations on the moss *Bryum capillare* Hedw (*BC*). Antimicrobial and antibiofilm activity were tested by MIC and microplate biofilm methods on antibiotic resistant bacteria. While the antioxidant activity of the extract was evaluated by DPPH, metal chelating, plasma lipid peroxidation and total phenolic content, the antigenotoxicity and cytotoxicity were established by Comet test and the WST-1 Cell proliferation assay kit respectively. The MIC values were found to be $\geq$ 125 $\mu g.mL^{-1}$ and a biofilm inhibition of 3–5% against only *S. epidermidis* was observed. Total phenolic compounds were determined as 23.26 mg/g. The results of DPPH assay, chelating and plasma lipid peroxidation activity were found to be 15%, 3% and 4% respectively. The extract was observed to decrease the affect of H_2O_2 that cause DNA damage. The *BC* was also determined 60 ± 5% anticancer activity against SKBR 3 and 76 ± 5% anticancer activity against HeLa cells, where this concentration had only 18 ± 5% cytotoxicity against MCF-12A cells. Also, these results have indicated the potential of *Bryum capillare* for the first time in novel natural compounds search.

1. Introduction

The history of medicinal plants use for the treatment of diseases is probably dates back to beginning of human life (Altuner, 2011). Most of the medicines available today are estimated to be originally derived from plant sources, which can be interpreted as the accumulation of the knowledge about medicinal plants occurring over thousands of years (Elibol et al., 2011). Higher plants, mosses, lichens, ferns, algae, seaweed and fungi are proposed as some of the sources for natural pharmaceuticals. Although vascular plants (tracheophytes) are widely used as a source of natural pharmaceuticals, only minute amount of the bryophytes have screened in terms of their anti-infective properties until now (Onbasli et al., 2011; Onbasli et al., 2019).

Bryophytes are non-vascular plants (Hallingback and Hodgetts, 2000) and they have a very simple structure compare to the higher plants. They occur in nearly every ecosystem on the Earth and are important especially in biogeochemical cycles (Vanderpoorten and Goffinet, 2009). Bryophytes are known to be a good source of metabolites, which have good biological activity (Krzaczkowski et al., 2009). As a result of this, they are good candidates for novel active pharmaceutical compound researches (Asakawa, 2000). There are several studies in the literature presenting the anti-infective potential of bryophytes against viruses, microfungi and bacteria, and antitumor activity against some cancer cells. In addition to those, they were successfully used as inhibitors of some enzymes important in metabolic pathways causing diseases and some other properties such as anti-platelet, antioxidant were also presented (Singh et al., 2006; Cheng et al., 2012).

The attention about investigation of the medicinal properties especially originated from plants is growing in last few decades. Even though several studies are present in the literature especially about the antibacterial and antifungal activities of bryophyte samples, very few reports on a wide range of therapeutic effects of bryophytes were published until now. The aim of this study is to determine several in vitro activities, such as anticancer, antigenotoxic, antibiofilm, antioxidant and antimicrobial, of a bryophyte, namely *Bryum capillare* (*BC*). To the best of our knowledge, these

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results present the first demonstration about the wide range of activities of this bryophyte sample collected from Turkey.

2. Materials and methods

2.1. Moss samples

Bryum capillare Hedw. (*BC*) samples were collected from Ilgaz Mountain, Çatören, Kastamonu, Turkey.

2.2. Extraction procedure

Extraction procedure defined previously by Canli et al. was used (Canli et al., 2016) and ground *BC* samples and active compounds dissolved in extraction solvent (ethanol, Merck, Germany) were seprated by a filtration process (Whatman No. 1). Extraction solvent was then evaporated at 30 °C after attaching the extract to a rotary evaporator (Heidolph). The remaining solution was lyophilized (Christ Alpha 1–2 LD) and residues were used for further analysis.

2.3. Microorganisms

Candida albicans ATCC 10231, *Staphylococcus epidermidis* ATCC 12,228 and *Staphylococcus aureus* ATCC 25,923 were used in the study. Test microorganisms were cultured as it was mentioned before (Canli et al., 2017).

2.4. Antimicrobial activity

A concentration range of 1–500 $\mu g.mL^{-1}$ extracts were obtained and MIC values were determined as it was mentioned previously (Altuner et al., 2011).

2.5. Antibiofilm activity

50, 100 and 150 $\mu L.mL^{-1}$ of extracts were used to test antibiofilm activity and the growth conditions, biofilm formation, screening and calculation of biofilm inhibition were done according to prevous studies (Stepanović et al., 2011; Saharkhiz et al., 2012).

2.6. Antioxidant activity

The antioxidant activity of 0.25, 0.50 and 1.00 $mg.mL^{-1}$ of extracts were determined and calculated by DPPH radical scavenging assay (Blois, 1958), metal chelating activity on ferrous ions (Fe^{+2}) (Oke et al., 2009) and plasma lipid peroxidation inhibitory assay (Rodriguez-Martinez and Ruiz-Torres, 1992). Total phenolic compounds (TPC) were determined by gallic acid equivalence (GAE) method (Rodriguez-Martinez and Ruiz-Torres, 1992; Singleton et al., 1999; Oke et al., 2009) and the vitamin content was analysed by HPLC.

2.7. Genotoxic activity

Extracts of 250 and 500 $\mu g.mL^{-1}$ were used in Comet assay [19], and 500 and 1000 $\mu g.mL^{-1}$ of extracts were used for testing antigenotoxic activity on human lymphocytes and image analysis of comet assay measurements were conducted as mentioned by previous researchers (Noroozi et al., 2009).

2.8. Cytotoxic activity

Human breast epithelial cells (MCF-12A, ATCC CRL-10782) (Vorster et al, 2012) human breast cancer cells (SKBR 3, ATCC HTB-30) (Quirantes-Piné et al, 2013) and human cervix cancer cells (HeLa, ATCC CCL-2) (Lu et al, 2010) were used for cytotoxicity tests and the cytotoxicity of 500 and 1000 $\mu g.mL^{-1}$ of extracts were determined according to the protocol provided by WST-1 Cell proliferation assay kit (Cayman).

2.9. Statistics

All test was conducted in triplicates and to determine the significance of difference ANOVA test was used ($p < 0.05$).

3. Results

3.1. Antimicrobial activity

In this study, antimicrobial activity of the extracts was identified by determining the minimum inhibitory concentrations (MICs). A concentration range of 1, 2, 4, 8, 16, 32, 63, 125, 250, 500 $\mu g.mL^{-1}$ was established against each microorganism and MIC values identified for each microorganism were given in Table 1. According to the results all MIC values of the extracts against three strains were $\geq$ 125 $\mu g.mL^{-1}$.

3.2. Antibiofilm activity

Biofilm formation capacities of tested strains and biofilm inhibition percentages of the extract against these three test strains are given in Table 2 and 3 respectively. According to the results given in Table 3 the extract presented 3% and 5% antibiofilm activity for 50 $\mu L.mL^{-1}$ and 100 $\mu L.mL^{-1}$ of the extract respectively against *S. epidermidis* (strong biofilm formation).

3.3. Antioxidant activity

Antioxidant activity of extract and positive control was determined by DPPH assay for a concentration range of 0.25–1.00 $mg.mL^{-1}$ (Fig. 1). Inhibition percentage of DPPH scavenging ability by the extract was found to be changing between 2% and 15%, where the highest inhibition was observed for 1.00 $mg.mL^{-1}$ concentration. The IC_{50} values, which are the concentiration inhibitting 50% of the extract and BHA are compared by DPPH assay and shown in Table 4. The IC_{50} value of *BC* extract (8.41 ± 0.12 $mg.mL^{-1}$) presented not very high scavenging activity in DPPH assay. On the other hand, Table 4 shows the extract's percentage of metal chelating activity. Our extract exhibited weak chelating ability with a IC_{50} value of 49.67 ± 0.32 $mg.mL^{-1}$. However, EDTA, which was used a positive control showed very good chelating activity, which is about 94% for the concentrations tested Also the capacity of the extract to inhibit lipid peroxidation was determined in our study too. The generation of malondialdehyde (MDA) was substantially controlled by the extract. The extract exhibited in high inhibition activity (33.56 ± 1.12%) against plasma lipid peroxidation (Table 4).

Antioxidants, which are natural, such as total phenols, tocopherols and ascorbic acid were found to be in the ethanolic extract

Table 1
The minimum inhibitory concentration (MIC $\mu g.mL^{-1}$) values of *BC* extract against test microorganism.

Strains	MIC ($\mu g.mL^{-1}$)
S. aureus ATCC 25,923	≥125
S. epidermidis ATCC 12,228	≥125
C. albicans ATCC 10,231	≥125

Values are expressed as mean ± standart deviation.

Table 2
Biofilm formation of tested strains.

Strains	Biofilm formation
S. aureus ATCC 25,923	+
S. epidermidis ATCC 12,228	+++
C. albicans ATCC 10,231	++

+: weak, ++: moderate, +++: strong.
Values are expressed as mean ± standart deviation.

Table 3
Biofilm inhibition percentages.

Strains	*BC* extract concentration		
	50 μL.mL^{-1}	100 μL.mL^{-1}	150 μL.mL^{-1}
S. aureus ATCC 25,923	0	0	x
S. epidermidis ATCC 12,228	3%	5%	x
C. albicans ATCC 10,231	0	0	x

0: No antibiofilm activity observed.
x: No growth was observed since the concentration showed antimicrobial activity.
Values are expressed as mean ± standart deviation.

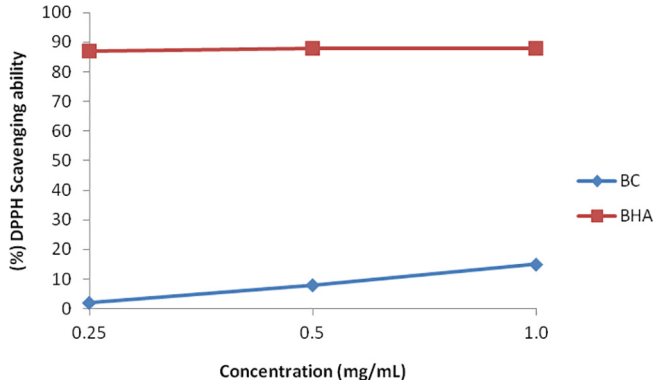

Fig. 1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the BC extract. Data are mean ± SD values for triplicate experiments. BHA, butylated hydroxyl anisole; *BC*, *Bryum capillare* ethanol extract.

Table 4
Antioxidant activities of *BC* extract.

Material	IC_{50} (mg.mL^{-1})		Plasma lipid peroxidation (%)
	DPPH	Metal chelating	
BC	8.41 ± 0.12	49.67 ± 0.32	33.56 ± 1.12
BHA	0.3 ± 0.1	NS	NS

[a]Data are average ± SD values for triplicate experiments. Values in the same column with different superscript upper case letters are significantly (p < 0.0.05) different.
IC_{50}, 50% inhibitory concentration.
NS, Not studied.
DPPH, 2,2-diphenyl-1-picrylhydrazyl
BHA (Buthylated hydroxyl anisole), Synthetic antioxidant.

of *BC* in our study. However, folic acid was not detected. The TPC determined for the extract was 23.26 mg GAE.g^{-1}. The extract was analysed by HPLC for their vitamin content (ascorbic acid, α-tocopherol and folic acid) and the total phenolic content which could cause the antioxidant activity. The data obtained from this analysis were given in Table 5. Ascorbic acid and α-tocopherol were found in lower amounts (1.87 and < 0.5 μg.g^{-1} respectively) in the extract.

3.4. Antigenotoxic and anticancer activities

The activity of *BC* extracts in terms of their genotoxicity was expressed in two points of views. Table 6 states, whether *BC* extracts presents genotoxic activity for human lymphocytes and Table 7 points out antigenotoxic activity results for *BC* extracts, which states the activity of the *BC* extract to inhibit the genotoxic activity of H_2O_2. According to Table 6, while 500 μg.mL^{-1} of *BC* extract caused no tail DNA damage, 1000 μg.mL^{-1} of *BC* extract presented only 0.05% of tail DNA damage, where positive control was the cause of 54% of tail DNA damage. On the other hand, according to Table 7, 500 μg.mL^{-1} and 1000 μg.mL^{-1} of *BC* extract inhibited the genotoxic activity of 50 μM H_2O_2 as 39% and 55% respectively. Also, *BC* extract presented ~ 30% decrease in tail length for 500 μg.mL^{-1} of the extract and ~ 45% decrease in tail length for 1000 μg.mL^{-1} (Fig. 2).

Table 5
The total phenolic content (mg GAE.g^{-1}), ascorbic acid, α - tocopherol and folic acid content (μg.mL^{-1}) of *BC* extract.

Compound	Content
Total phenolic content	23,26
Ascorbic acid	1.87
α - tocopherol	<0.5
Folic acid	ND

Values are expressed as mean ± standart deviation.
ND, not determined.

Three different cell lines, MCF-12A (human breast epithelial cells), SKBR 3 (human breast cancer cells) and HeLa (human cervix cancer cells), with two different concentrations (500 μg.mL^{-1} and 1000 μg.mL^{-1}) were used in the study. Cytotoxicity test results in terms of cell viability (%) are given in Fig. 3. According to Fig. 3 survival rate for a mammalian cell, human breast epithelial cells (MCF-12A) was 100% and 82% after incubation with 500 μg.mL^{-1} and 1000 μg.mL^{-1} *BC* extracts respectively. But for human breast cancer cells (SKBR 3) the survival rate was decreased to 69% and 40% after incubation with 500 μg.mL^{-1} and 1000 μg.mL^{-1} *BC* extracts respectively. These rates were much lower in human cervix cancer cells (HeLa) such as 67% and 24% after incubation with 500 μg.mL^{-1} and 1000 μg.mL^{-1} *BC* extracts respectively. Furthermore, cytotoxicity results given in Fig. 3 presented that the extract of 500 μg.mL^{-1} is also safe, since the cytotoxicity rate of this extract was 0% on MCF-12A cells. Instead, as it was stated in Table 6 the extract of 1000 μg.mL^{-1} caused only 0.05% of DNA damage and the cytotoxicity rate of this concentration was 18 ± 5% on MCF-12A cells (Fig. 3). According to the results it can be concluded that *BC* extract presented notable anticancer activity against SKBR 3 and HeLa cells, while having relatively very low cytotoxic activity against MCF-12A cells.

4. Discussion

Plants have the ability to synthesize aromatic substances such as phenolic and nitrogen containing compounds vitamins, terpenoids and some other endogenous metabolites, which are active against herbivores, insects, bacteria and fungi (Bharathi et al., 2011). It is known that as a result of extensive use and in addition misuse of antibiotic and antifungal agents led to multi drug resistance (MDR). Thus, there is a need to discover novel antibacterial and antifungal agents from different sources and plants are one of them (Karaman et al., 2003). It was previously proposed that bryophytes are good candidates to discover potential active compounds (Zhu et al., 2006). Some bryophytes have ethnobotanical uses against several diseases, such as skin infections (Veljić, et al., 2009). There are several results regarding the antibacterial and antifungal activities of bryofite extracts in the literature (Singt and Rawat, 2006; Vidal et al., 2012; Bukvički et al., 2012; Onbasli et al., 2019). In this study, antimicrobial activity of the

Table 6
Genotoxic activity of *BC* extracts for human lymphocytes.

Sample	Concentration	Tail Length (μm)	Tail Moment (μm)	Tail DNA Damage (%)
Negative Control	–	28.8 ± 0.02	0.00 ± 0.00	0.00
Positive Control (H_2O_2)	50 μM	74.0 ± 1.95	18.00 ± 0.001	54
BC extract	500 μg.mL^{-1}	28.96 ± 0.27	0.00 ± 0.00	0.00
BC extract	1000 μg.mL^{-1}	29.46 ± 4.30	0.26 ± 0.06	0.05

Values are expressed as mean ± standart deviation.

Table 7
Antigenotoxic activity of *BC* extracts for human lymphocytes.

Sample	Concentration	Tail Length (μm)	Tail Moment (μm)	Tail DNA Damage (%)	Inhibiton (%)
Negative Control	–	28.8 ± 0.02	0.00 ± 0.00	0.00	–
Positive Control (H_2O_2)	50 μM	74.0 ± 1.95	18.00 ± 0.001	54.0	–
BC extract + H_2O_2	500 μg.mL^{-1}	52.05 ± 0.27	11.00 ± 0.00	33.00	39
BC extract + H_2O_2	1000 μg.mL^{-1}	41.03 ± 3.30	8.26 ± 0.00	25.05	55

Values are expressed as mean ± standart deviation.

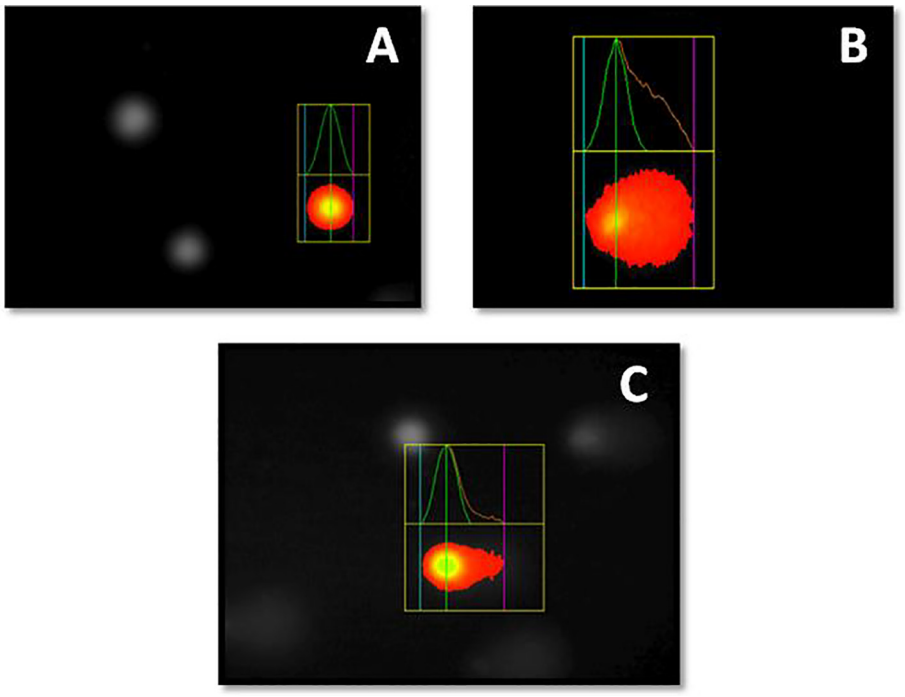

Fig. 2. A. Negative control: Healthy human lymphocytes, B. Positive control: Human lymphocytes incubated with 50 μM H_2O_2, C. Human lymphocytes incubated with *BC* extracts.

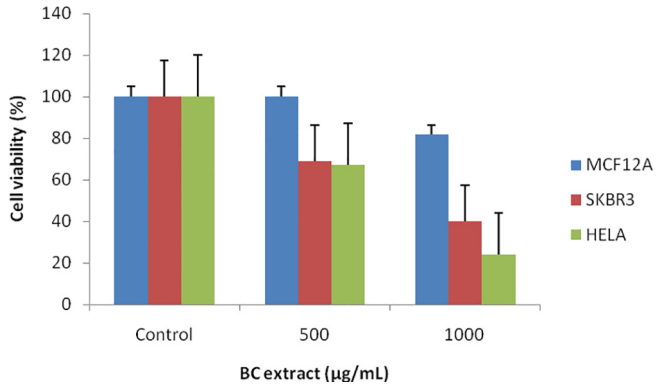

Fig. 3. Cytotoxic effect of the *BC* extract in MCF12A, SKBR3 and HELA cells pretreated with the extract at different concentrations (500 μg.mL^{-1} and 1000 μg.mL^{-1}) for 48 h. Each value represents the mean ± SD for triplicate experiments.

extracts was identified by determining the minimum inhibitory concentrations (MIC) and MIC values identified for each microorganism were ≥ 125 μg.mL^{-1}. Previously the antibacterial and antifungal activity of *BC* methanol extract were tested against *Bacillus megaterium*, *Shigella flexneri*, *Salmonella enterica* serotype *Typhimirium* SL 1344, *Escherichia coli* ETEC LM 63,083 and *Candida albicans* ATCC 26,555 by MIC. They were found that the methanol extract of this bryophyte was active against *S. flexneri* and *B. megaterium* and the MIC values were 20 mg.mL^{-1} for both microorganism. However, the ethanol extract of *Cratoneuron filicinum* presented antibacterial activity against six bacteria with a MIC value ranging between 1.56 and 3.12 μg.mL^{-1} and antifungal activity with a MIC value of 3.12 μg.mL^{-1} (Singt and Rawat, 2006). In addition, ethanolic extract of *Octoblepharum albidum* was previously tested for its antibacterial activity against six microorganisms by MIC test and an activity against *Klebsiella pneumoniae* ATCC 33,018 and *E. coli* ATCC 25,922 was observed with a MIC value of 512 μg.mL^{-1}

(Vidal et al., 2012). Onbasli et al. (2019) were tested *Hypnum andoi* extracts against skin pathogens, because bryophytes have ethnobotanical uses against several diseases, such as skin infections (Bukvički et al., 2012). Antimicrobial activity of *BC* is higher than *Hypnum andoi*. Our study is supported by the studies present in the literature.

Biofilms have been reported by several researchers that they involve in several types of infections mostly related with some permenant indwelling devices such as heart valves, joint prostheses and catheters, but also found to cause endocarditis, dental plaque formation, middle-ear infections and urinary tract infections (Parsek and Singh, 2003). As a reason of this, scientists are trying to determine compounds those would inhibit the formation of biofilms. There are no studys in the literature either about the antibiofilm activity of *BC* or detailed studies regarding the antibiofilm activity of mosses. Thus, it is not possible to compare our results with any other bryophyte sample. Biofilm formation capacities of tested strains and biofilm inhibition percentages of the extract against these three test strains were highest found 3% and 5% antibiofilm activity for 50 $\mu L.mL^{-1}$ and 100 $\mu L.mL^{-1}$ of the extract respectively against *S. epidermidis*. It is known that biofilms are also form on biotic surfaces such as human skin (Karatan and Watnick, 2009) and *S. epidermidis* is one of the microorganisms, which are responsible for biofilm formation on skin. All microorganisms used in this study have capability of causing formation of biofilms on human skin (Nostro et al., 2007; Sudjana et al., 2012; Onbasli et al., 2019). The biofilm inhibition percentages of the extracts were found to be very low, when compared to other plant extracts, such as *Boswellia papyrifera* oils, which presented 71% to 99% inhibition against *S. epidermidis* with concentrations between 217 $\mu g.mL^{-1}$ and 6.8 $\mu g.mL^{-1}$ (Schillaci et al., 2008). But the results regarding *BC* extract are very promising, since increasing concentration would possibly increase the biofilm inhibition percentages against *S. epidermidis*.

Today it is known that free radicals often damage biomolecules leading to cellular senescence, cancer and even cell death (Mantle et al., 1998). The best way to reduce the amount of free radicals is using compounds presenting antioxidant activities (Souri et al., 2008). Exogenous and endogenous antioxidants can be used to prevent free radical formation (Cesquini et al., 2003). As a reason of this, studies about novel antioxidants from natural origins are under investigation. Plants are one of the sources for natural antioxidants. Although no reports have been published about antioxidant acitivities of *BC*, several studies regarding the antioxidant activity of other bryophytes are available (Dey and De, 2012; Onbasli et al., 2019). Antioxidants, which are natural, such as total phenols, tocopherols and ascorbic acid were found to be in the ethanolic extract of *BC* in our study. However, folic acid was not detected. Ascorbic acid and α-tocopherol were found in lower amounts (1.87 and < 0.5 $\mu g.g^{-1}$ respectively) in the extract. Thus, the antioxidant activity can be related to the total phenols. The TPC value is higher than some of the mosses which were analysed by Chobot et al. (2006), Pejin and Bogdanovic-Pristov (2012) and Mukhopadhyay et al. (2013). But when the IC_{50} values for antioxidant activity were analysed by comparing the results published by previous studies, it is obvious that IC_{50} values were quite high for *BC* extract. For example, IC_{50} values were previously found to be between 0.170 ± 0.001 $mg.mL^{-1}$ and 0.487 ± 0.001 $mg.mL^{-1}$ for *M. marginatum*, *L. glaucum*, *D. scoparium*, *D. polysetum* and *C. purpureus* by DPPH assay (Chobot et al., 2006), where in our study this value is 8.41 $mg.mL^{-1}$. The main question which can be posed here for the extract could be about its relatively low antioxidant activity with having a relatively average TPC content. Chobot et al. (2006) has also observed such a case and concluded that there is no significant correlation with TPC concentration and antioxidant activity ($\alpha = 0.05$). As a result, they pointed out that antioxidant activity can be modulated by several components having antagonistic or synergistic activities. Our results also supported this observation.

Most people believe that pharmaceutical plants are harmless when compared to the synthetic drugs (Celik, 2017), but scientists proved that most of the plants having enthnopharmalogical uses have a potential of being cytotoxic, genotoxic and carcinogenic (Ferreira-Machado et al., 2004). In this context, any natural product presenting anticancer properties should be tested for their toxicity potential. Anticancer properties of mosses have been studied by several researchers (Onbasli et al., 2019; Yağlıoğlu et al., 2017); however no detailed reports have been published until now either about the toxicity of *BC* or the toxicity of other mosses in the literature. For this reason, the positive anticancer properties of mosses published until now include false-positive potentials and in addition these studies are lack of toxicity information (Cos et al., 2006). Antigenotoxic activity of natural products has been increasingly interested in by researchers in last few decades (Hayder et al., 2004) because such products are thought to be beneficial against some mutation-related diseases and cancer (De Flora, 1998). There are several studies regarding antigenotoxic properties of some plants, but all of them are for higher plants. Marques et al. (2011) analysed the antigenotoxic activity of *Ginkgo biloba* leaf extract in *Saccharomyces cerevisiae*. Hayder et al. (2004) studied the antigenotoxic activity of *Myrtus communis* in *E. coli* PQ37 and Leite-Silva et al. (2007) used *Fucus vesiculosus* to observe its antigenotoxic activity in human lymphocytes. Marques et al. (2011) observed ~85% decrease in tail length compared to positive control (10 mM H_2O_2) after incubating yeast cells by a mixture of H_2O_2 and *G. biloba* extract. *BC* extract presented ~ 30% decrease in tail length for 500 $\mu g.mL^{-1}$ of the extract and ~ 45% decrease in tail length for 1000 $\mu g.mL^{-1}$. The extract is also observed to lower the affect of H_2O_2 on human lymphocytes that cause DNA damage. As a conclusion it is possible to propose further *in vivo* tests to be conducted especially for 500 $\mu g.mL^{-1}$ of the extract. Hayder et al. (2004) found up to 99% inhibition of genotoxicity by *Myrtus communis* extracts depending on the concentration and solvent used for extraction. The inhibition of genotoxicity by the extract was 39% for 500 $\mu g.mL^{-1}$ and 55% for 1000 $\mu g.mL^{-1}$. Onbasli et al. (2019) used *Hypnum andoi* to observe its antigenotoxic activity in human lymphocytes. The inhibition of genotoxicity by the extract was 35% for 500 $\mu g.mL^{-1}$ and 53% for 1000 $\mu g.mL^{-1}$. According to the results it is obvious that *BC* extract has some antigenotoxic and anticancer potentials. Although the results are still promising, according to the genotoxicity and cytotoxicity tests it is possible to propose an increase in the concentration up to 5 folds for better anti-infective activity. It is known that *S. epidermis* has a potential of causing skin infections. It is possible to recommend that with an increase in the concentration, *BC* extract can be used in cosmetics that could have a potential of inhibiting skin infections caused by biofilm formed by *S. epidermis*. According to the results it can be concluded that *BC* extract presented notable anticancer activity against SKBR 3 and HeLa cells, while having relatively very low cytotoxic activity against MCF-12A cells. The extract is also observed to lower the affect of H_2O_2 on human lymphocytes that cause DNA damage. As a conclusion it is possible to propose further *in vivo* tests to be conducted especially for 500 $\mu g.mL^{-1}$ of the extract (Fig. 2).

5. Conclusions

Our data for the first time, demonstrated the promising antimicrobial, antibiofilm, antioxidant, antigenotoxic and anticancer activities of *Bryum capillare*, a bryophyte sample in searching for novel therapeutic agents. It may be used as alternative natural sources applications in agriculture, cosmetic and drug industries. However, further phytochemical, experimental and clinical investigations will show more of its medicinal potentials.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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References

Altuner, E.M., 2011. Investigation of antimicrobial activity of *Punica granatum* L. fruit peel ash used for protective against skin infections as folk remedies especially after male circumcision. Afr. J. Microbiol. Res. 5 (20), 3339–33342.
Altuner, E.M., Çeter, T., Bayar, E., Aydın, S., Arıcı, F., Suleymanoglu, G., Edis, A., 2011. Investigation on antimicrobial effects of some moss species collected from Kastamonu region. Commun. Fac. Sci. Univ. Ank. Series C. 23 (1–2), 33–43.
Asakawa, Y., 2000. Biologically active compounds from bryophytes. Pure Appl. Chem. 79, 557–580.
Bharathi, V., Shanmuga-Priya, A., Firdous, S.J., 2011. Antibacterial activity of stem extracts of *Ocimum basilicum*. J. Chem. Biol. Physic. Sci. 2 (1), 298–301.
Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature 181, 1199–2000.
Bukvički, D., Veljić, M., Soković, M., Grujić, S., Marin, P.D., 2012. Antimicrobial activity of methanol extracts of *Abietinella abietina*, *Neckera crispa*, *Platyhypnidium riparoides*, *Cratoneuron filicinum* and *Campylium protensum* mosses. Arch. Biol. Sci. Belgrade. 64 (3), 911–916.
Canli, K., Akata, I., Altuner, E.M., 2016. In vitro antimicrobial activity screening of *Xylaria hypoxylon*. Afr. J. Tradit. Complement. Altern. Med. 13 (4), 42–46.
Canli, K., Yetgin, A., Akata, I., Altuner, E.M., 2017. Antimicrobial activity and chemical composition screening of *Epilobium montanum* root. Indian J. Pharm. Educ. Res. 51, 239–243.
Celik, T.A., 2017. Potential genotoxic and cytotoxic effects of plant extracts, in: Bhattacharya, A. (Eds.), A compendium of essays on alternative therapy, In Tech, pp. 233-250.
Cesquini, M., Torsoni, M.A., Stoppa, G.R., Ogo, S.H., 2003. t-BOOH-induced oxidative damage in sickle red blood cells and the role of flavonoids. Biomed. Pharmacother. 57, 124–129.
Cheng, X., Xiao, Y., Wang, X., Wang, P., Li, H., Yan, H., Liu, Q., 2012. Anti-tumor and pro-apoptic activity of ethanolic extract and its various fractions from *Polytrichum commune* L. Ex Hedw in L1210 cells. J. Ethnopharmacol. 143, 49–56.
Chobot, V., Kubicova, L., Nabbout, S., Jahodar, L., Vytlacilova, J., 2006. Antioxidant and free radical scavenging activities of five moss species. Fitoterapia 77, 598–600.
Cos, P., Vlietinck, A.J., Vanden Berghe, D., Maesa, L., 2006. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. J. Ethnopharmacol. 106, 290–302.
De Flora, S., 1998. Mechanisms of inhibitors of mutagenesis and carcinogenesis. Mut. Res. 420, 151–158.
Dey, A., De, J.N., 2012. Antioxidative potential of Bryophytes: Stress tolerance and commercial perspectives: A review. Pharmacologia. 3, 151–159.
Elibol, B., Ezer, T., Kara, R., Yuvali Çelik, G., Çolak, E., 2011. Antifungal and antibacterial effects of some acrocarpic mosses. Afr. J. Biotechnol. 10 (6), 986–989.
Hallingback, T., Hodgetts, N., 2000. Status survey and conservation Action plan for Bryophytes: mosses, liverworts and hornworts. IUCN/SSC Bryophyte Specialist Group, Switzerland and Cambridge.
Hayder, N., Abdelwaheda, A., Kilani, S., Ammar, R.B., Mahmoud, A., Ghedira, K., Chekir-Ghedira, L., 2004. Anti-genotoxic and freeradical scavenging activities of extracts from (Tunisian) Myrtus communis. Mutat. Res. 564, 89–95.
Ferreira-Machado, S.C., Rodrigues, M.P., Nunes, A.P., Dantas, F.J., de Mattos, J.C., Silva, C.R., Moura, E.G., Bezerra, R.J., Caldeira-de-Araujo, A., 2004. Genotoxic potentiality of aqueous extract prepared from *Chrysobalanus icaco* L. leaves. Toxicol. Lett. 151 (3), 481–487.
Karaman, İ., Şahin, F., Güllüce, M., Ogütcü, M.H., Sengül, M., Adıguzel, A., 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol. 85, 231–235.
Karatan, E., Watnick, P., 2009. Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol. Mol. Biol. Rev. 73, 310–347.
Krzaczkowski, L., Wright, M., Reberioux, D., Massiot, G., Etievant, C., Gairin, J.E., 2009. Pharmacological screening of bryophyte extracts that inhibit growth and induce abnormal phenotypes in human HeLa cancer cells. Fund. Clin. Pharmacol. 23, 473–482.
Leite-Silva, C., Gusmão, C.L.S., Takahashi, C.S., 2007. Genotoxic and antigenotoxic effects of *Fucus vesiculosus* extract on cultured human lymphocytes using the chromosome aberration and Comet assays. Genet. Mol. Biol. 30, 105–111.
Lu, B., Hu, M., Liu, K., Peng, J., 2010. Cytotoxicity of berberine on human cervical carcinoma HeLa cells through mitochondria, death receptor and MAPK pathways, and in-silico drug-target prediction. Toxicol. In Vitro 24, 1482–1490.
Mantle, D., Anderton, J.G., Falkous, G., Barnes, M., Jones, P., Perry, E.K., 1998. Comparison of methods for determination of total antioxidant status: application to analysis of medicinal plant essential oils. Comp. Biochem. Phys. B. 121, 385–391.
Marques, F., Azevedo, F., Johansson, B., Oliveira, R., 2011. Stimulation of of DNA repair in *Saccharomyces cerevisiae* by *Ginkgo biloba* leaf extract. Food Chem. Toxicol. 49, 1361–1366.
Mukhopadhyay, S.T., Mitra, S., Biswas, A., Das, N., Poddar-Sarkar, M., 2013. Screening of Antimicrobial and Antioxidative Potential of Selected Eastern Himalayan Mosses. Eur. J. Med. Plant. 3 (3), 422–428.
Noroozi, S., Mosaffa, F., Soltani, F., Iranshahi, F.M., Karimi, G., Malekaneh, M., Haghighi, F., Behravan, J., 2009. Antigenotoxic effects of the disulfide compound persicasülfide A (PSA) on Rat Lymphocytes exposed to oxidative stress. Planta Med. 75, 32–36.
Nostro, A., Sudano, R.A., Bisignano, G., Marino, A., Cannatelli, M.A., Pizzimenti, F.C., Cioni, P.L., Procopio, F., Blanco, A.R., 2007. Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. J. Med. Microbiol. 56, 519–523.
Oke, F., Aslim, B., Ozturk, S., Altundag, S., 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem. 112, 874–879.
Onbasli, D., Altuner, E.M., Yuvali Çelik, G., 2011. *Mnium marginatum* Özütlerinin Antimikrobiyal Aktivitesi. Kastamonu Ün. Orman Fak. Derg. 11 (2), 205–208.
Onbasli, D., Yuvalı, Celik, G., Altuner, E.M., Aslim, B., 2019. Investigation of Pharmacological Properties of Bryophyte Hypnum andoi from Turkey. Int. Pharm. Nat. Med. 7(1), 10-14.
Parsek, M.R., Singh, P.K., 2003. Bacterial biofilms: an emerging link to disease pathogenesis. Annu. Rev. Microbiol. 57, 677–701.
Pejin, B., Bogdanovic-Pristov, J., 2012. ABTS cation scavenging activity and total phenolic content of three moss species. Hem. Ind. 66 (5), 723–726.
Quirantes-Piné, R., Zurek, G., Barrajón-Catalá, E., Bäßmann, C., Micol, V., Segura-Carretero, A., Fernàndez-Gutièrrez, A., 2013. A metabolite-profiling approach to assess the uptake and metabolism of phenolic compounds from olive leaves in SKBR3 cells by HPLC–ESI-QTOF-MS. J. Pharmaceut. Biomed. 72, 121–126.
Rodriguez-Martinez, M.A., Ruiz-Torres, A., 1992. Homeostasis between lipid peroxidation and antioxidant enzyme activities in healthy human aging. Mech. Ageing Dev. 6, 213–222.
Saharkhiz, M.J., Motamedi, M., Zomorodian, K., Pakshir, K., Miri, R., Hemyari, K., 2012. Chemical Composition, Antifungal and Antibiofilm Activities of the Essential Oil of *Mentha piperita* L. ISRN Pharma., 1–6
Schillaci, D., Arizza, V., Dayton, T., Camarda, L., Di Stefano, V., 2008. In vitro anti-biofilm activity of *Boswellia* spp. oleogum resin essential oils. Lett. Appl. Microbiol. 47 (5), 433–438.
Singh, M., Govindarajan, R., Nath, V., Rawat, A.K.S., Mehrotra, S., 2006. Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. J. Ethnopharmacol. 107, 67–72.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Method Enzymol. 99, 152–178.
Singt, M., Rawat, A.K.S., 2006. Govindarajan R. Antimicrobial activity of some Indian mosses. Fitoterapia 78, 156–158.
Souri, E., Amin, G., Farsam, H., Jalalizadeh, H., Barezi, S., 2008. Screening of Thirteen Medicinal Plant Extracts for Antioxidant Activity. Iranian J. Pharmaceutic. Res. 7 (2), 149–154.
Stepanović, S., Vuković, D., Ježek, P., Pavlović, M., Švabić-Vlahović, M., 2011. Biofilm formation by staphylococci under static and dynamic conditions. Eur. J. Clin. Microbiol. Infect. Dis. 20, 502–504.
Sudjana, A.N., Carson, C.F., Carson, K.C., Riley, T.V., Hammer, K.A., 2012. *Candida albicans* adhesion to human epithelial cells and polyst yrene and formation of biofilm is reduced by sub-inhibitory *Melaleuca alternifolia* (tea tree) essential oil. Med. Mycol. 50, 863–870.
Vanderpoorten, A., Goffinet, B., 2009. Introduction to bryophytes. Cambridge University Press, Cambridge.
Veljić, M., Đurić, A., Soković, M., Ćirić, A., Glamočlija, J., Marin, P.D., 2009. Antimicrobial Activity of methanol extracts of *Fontinalis antipyretica*, *Hypnum cupressiforme*, and *Ctenidium molluscum*. Arch. Biol. Sci. Belgrade. 61 (2), 225–229.
Vidal, C.A.S., Souza, E.O., Rodrigues, F.F.G., Campos, A.R.S., Lacerda, R., Costa, J.G.M., 2012. Phytochemical Screening and Synergistic Interactions between Aminoglycosides, selected antibiotics and extracts from the bryophyte *Octoblepharum Albidum* Hedw (Calymperaceae). Arch. Biol. Sci. Belgrade. 64 (2), 465–470.
Vorster, C., Stander, A., Joubert, A., 2012. Differential signaling involved in Sutherlandia frutescens-induced cell death in MCF-7 and MCF-12A cells. J. Ethnopharmacol. 140, 123–130.
Yağlıoğlu, M.S., Abay, G., Demirtas, I., Sahin Yağlıoğlu, A., 2017. Phytochemical screening, antiproliferative and cytotoxic activities of the mosses Rhytidiadelphus triquetrus (Hedw.) Warnst. and Tortella tortuosa (Hedw.) Limpr. Anatolian Bryol. 3 (1), 31–42.
Zhu, R.L., Wang, D., Xu, L., Shi, R.P., Wang, J., Zeng, M., 2006. Antibacterial activity in extracts of some bryophytes from China and Mongolia. J. Bot. Lab. 100, 603–615.