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INVESTIGATION ON ANTIMICROBIAL EFFECTS OF SOME MOSS SPECIES COLLECTED FROM KASTAMONU REGION

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ABSTRACT

It has been known for centuries that mosses are effective in wound healing process. Previous studies presented that some secondary metabolites of mosses have anti-infective effect on some microorganisms.

In this study, *in vitro* antimicrobial effect of *Bryum capillare* var. *capillare*, *Plagiomnium affine*, *Homalothecium sericeum*, *Homalia besseri*, *Amblystegium tenax* and *Homalothecium lutescens* samples collected from Kastamonu region were examined against *Candida albicans* ATCC 26555, *Salmonella enterica* Serotype Typhium SL 1344, *Escherichia coli* ETEC LM 63083, *Shigella flexneri* and *Bacillus megaterium*. The antimicrobial activities of moss extracts were evaluated by disc diffusion method and the results were supported MIC (minimum inhibitory concentration) and MBC/MFC (minimum bactericidal/fungicidal concentration) tests.

According to the results, among the moss samples, *Bryum capillare* var. *capillare* presented an antimicrobial activity against *S. flexneri* and *B. megaterium*, where *Homalothecium sericeum* and *Amblystegium tenax* had an antimicrobial activity against only *S. flexneri*, and *Homalia besseri* against only *B. megaterium*.

KEYWORDS: Mosses, antimicrobial activity, disc diffusion method, MIC, MBC, MFC

INTRODUCTION

Most of the plants have an almost limitless ability to synthesize aromatic substances, most of which are in phenolic nature or their derivatives (Geissman, 1963). These substances are often used as a defence mechanism against microorganisms, insects and herbivores (Samidurai and Saravanakumar, 2009).

The antimicrobial activity of plants has many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997; Reynolds, 1996).

In contrast to the extensive utilisation of higher plants as a source of antimicrobial substances, Bryophytes have rarely been considered for this (Basile, Vuotto, Ielpo, Moscattiello, Ricciardi, Giordano and Cobianchi, 1998).

It has been known for centuries that mosses are effective in both wound healing process and lowering the risk of infection. In the previous studies it has been presented that some secondary metabolites extracted from mosses have anti-infective effect on some bacteria and fungi (Altuner, 2008; Altuner and Çetin, 2009; Altuner and Çetin, 2010; Altuner, Çetin and Çökmüş, 2010a; Altuner, Çetin and Çökmüş, 2010b; Veljic, Tarbuk, Marin, Ciric, Sokovic and Marin, 2008; Basile, Vuotto, Ielpo, Moscattiello, Ricciardi, Giordano and Cobianchi, 1998).

Several bryophyte samples were used by Chinese Traditional Medicine to treat cardiovascular diseases, tonsillitis, bronchitis, cystitis and skin infections. North American Indians were used some bryophytes, such as *Bryum*, *Mnium* and *Philonotis*, to heal burns, bruises and wounds (Saroya, 2011).

Since drug resistance develops in human pathogens against commonly used antibiotics, there is a need for a search about new antimicrobial substances from other sources including plants (Erdogrul, 2002).

In this study, *in vitro* antimicrobial effect of *Bryum capillare* Hedw. var. *capillare*, *Plagiomnium affine* (Blandow ex Funck) T.J. Kop., *Homalothecium sericeum* (Hedw.) Schimp., *Homalia besseri* (Blandow ex Funck) T.J. Kop., *Amblystegium tenax* (Hedw.) C.E.O. Jensen and *Homalothecium lutescens* (Hedw.) H. Rob. samples collected from Kastamonu region were examined against *Candida albicans* ATCC 26555,

Salmonella enterica Serotype Typhium SL 1344, *Escherichia coli* ETEC LM 63083, *Shigella flexneri* and *Bacillus megaterium*.

MATERIALS AND METHODS

Moss Samples

Bryum capillare Hedw. var. *capillare*, *Plagiomnium affine* (Blandow ex Funck) T.J. Kop., *Homalothecium sericeum* (Hedw.) Schimp., *Homalia besseri* (Blandow ex Funck) T.J. Kop., *Amblystegium tenax* (Hedw.) C.E.O. Jensen and *Homalothecium lutescens* (Hedw.) H. Rob. samples collected from Kastamonu, TURKEY. The identification process was carried out by Kerem Canlı. The locations were given in Table 1.

Table 1. Localisations of the moss samples

MOSS SAMPLE	LOCATION
<i>Bryum capillare</i> var. <i>capillare</i>	Kastamonu, Ilgaz Mountain, Çatören.
<i>Plagiomnium affine</i>	Kastamonu, Küre Mountain.
<i>Homalothecium sericeum</i>	Kastamonu, Set Alabalık Çiftliği.
<i>Homalia besseri</i>	Kastamonu, Yaralıgöz Mountain.
<i>Amblystegium tenax</i>	Kastamonu, Küre, Çatak Dam.
<i>Homalothecium lutescens</i>	Kastamonu, İnebolu.

Extraction Solvent

Altuner (2008) stated that sterile distilled H₂O (sdH₂O), ethanol, methanol, chloroform, benzene, diethyl ether and ethyl acetate can be used to extract active substances.

Cowan (1999) proposed that most of the active substances are in aromatic or saturated organic nature. Therefore, these substances can easily be extracted by ethanol or methanol. For this reason methanol (anhydrous) (Sigma-Aldrich, Germany) was chosen in the study.

Microorganisms

Candida albicans ATCC 26555, *Escherichia coli* ETEC LM 63083, *Salmonella enterica* serotype Typhimurium SL 1344, *Shigella flexneri* (clinical isolate) and *Bacillus megaterium* (soil isolate) were used in the

study (Kastamonu University, Department of Biology, Botanical Research Laboratory Culture Collection).

Preparation of Inocula

All bacterial strains were incubated in atmospheric air at 37°C for 24 hours and *C. albicans* at 27°C for 48 hours. Inocula were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standard having approximately 10^8 cfu.mL⁻¹ for bacteria and 10^7 cfu.mL⁻¹ for *C. albicans* (Hammer, Carson and Riley, 1999). Mueller-Hinton Agar (Merck, Germany) medium is used for bacteria, where *C. albicans* strain was plated on Sabouraud Dextrose Agar (Merck, Germany).

Extraction Procedure

Moss samples were pulverised by using mortar and pestle. Pulverised samples were extracted in methanol by shaking at room temperature for 24 hours. Extracts were filtrated after 24 hours by using Whatman No 1 filter paper. Filtrates were evaporated by a rotary evaporator at 30°C and pulverised by a freeze-dryer. The pulverised residues were used to prepare extracts having 150 mg.mL⁻¹ concentrations.

Disc Diffusion Method

Disc diffusion test was performed as described previously by Andrews (2007). The culture medium was poured into 120 mm sterile Petri dish to give a mean depth of 4.0 mm \pm 0.5 mm (Altuner and Çetin, 2009). 10 µl, 20 µl and 30 µl aliquots of each extract were applied on sterile paper discs of 6 mm diameter (Mahasneh and El-Oqlah, 1999). To get rid of any residual solvent which might interfere with the results, discs were left to dry overnight at 30°C in sterile conditions (Silici and Koc, 2006). The surface of the plates was inoculated using previously prepared inocula containing saline suspension of microorganisms. Inoculated plates were then left to dry for 5 minutes at room temperature before applying the discs. Discs were firmly applied to the surface of the plate which had an even contact with the agar. Plates were incubated and inhibition zone diameters were expressed in millimetres.

Determination of MIC

Broth dilution method for Minimum Inhibitory Concentration (MIC) determination as described in Basile et al. (1998) was performed. Serial 2-fold dilutions were made to obtain a concentration range of 0.0039 - 2 mg.mL⁻¹. The MIC was defined as the lowest concentration of extract inhibiting any visible bacterial growth.

Determination of MBC and MFC

The Minimum Bactericidal Concentration (MBC) and the Minimum Fungicidal Concentration (MFC) determination were performed by sub-culturing suspensions from non-turbid MIC test tubes to agar medium. The MBC and MFC values were defined as the lowest concentration of extract inhibiting bacterial and fungal growth.

Controls

All extraction solvents and empty sterile discs were used as negative controls.

Statistics

The data determined as the mean of 3 parallel studies. All values given here are mean values of these 3 parallel studies.

The statistical analysis was performed using a non-parametric method Kruskal-Wallis one-way analysis of variance. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The aim of this study was to investigate the antimicrobial activity of some moss samples collected from Kastamonu region. To do this, the first test performed was disc diffusion test. In this test, extracts were loaded on empty sterile discs and these discs were then applied on a culture medium inoculated with microorganisms. If the extracts were active against microorganisms, they have caused an inhibition zone. The diameters of the inhibition zones recorded as the diameters of the zones in millimetres are given in Table 2.

Antimicrobial substances may have lethal or static type of activity. Lethal agents have a capability of killing microorganisms, where static agents have

a capability of inhibiting the growth or reproduction of microorganisms. The disc diffusion test alone is not enough to decide whether the activity type is lethal or static. In order to identify the type of the activity the disc diffusion test should be followed by MIC and MBC/MFC tests. Lethal agents have MFC values that are close to the MIC values. For static agents, the MIC values are much lower than the MFC values.

The MIC values, which were defined as the lowest concentration of extract inhibiting any visible microorganism growth stated as mg.mL^{-1} are given in Table 3. The MBC and MFC values which were defined as the lowest concentration of extract inhibiting bacterial and fungal growth after sub-culturing suspensions from non-turbid MIC test tubes to agar medium stated as mg.mL^{-1} are given in Table 3.

According to these results, *Bryum capillare var. capillare* showed antimicrobial activity on *S. flexneri* and *B. megaterium*, *Homalothecium sericeum* and *Amblystegium tenax* on only *S. flexneri* and *Homalia besseri* on only *B. megaterium*.

DISCUSSION AND CONCLUSION

When the results of disc diffusion test are examined, it can be seen that the values are not very high. This could be because of keeping the stock concentrations very low at the beginning.

When disc diffusion test results are interpreted by using the MIC and MBC/MFC values, it can be concluded that only the activity of *Bryum capillare var. capillare* on *B. megaterium*, was lethal type of activity. All of the others were static type of activity.

But these results should be supported by further large-scale studies, which will emphasize mainly the characterization of antimicrobial agents and their mechanism of action.

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Table 2. Disc Diffusion Test Results (Inhibition zones - mm)

		<i>Bryum capillare</i> var. <i>capillare</i>	<i>Plagiomnium</i> <i>affine</i>	<i>Homalothecium</i> <i>sericeum</i>	<i>Homalia besseri</i>	<i>Amblystegium</i> <i>tenax</i>	<i>Homalothecium</i> <i>lutescens</i>
<i>C. albicans</i>	10µl	-	-	-	-	-	-
	20µl	-	-	-	-	-	-
	30µl	-	-	-	-	-	-
<i>S. enterica</i>	10µl	-	-	-	-	-	-
	20µl	-	-	-	-	-	-
	30µl	-	-	-	-	-	-
<i>E. coli</i>	10µl	-	-	-	-	-	-
	20µl	-	-	-	-	-	-
	30µl	-	-	-	-	-	-
<i>S. flexneri</i>	10µl	-	-	-	-	-	-
	20µl	-	-	-	-	-	-
	30µl	8	-	10	-	10	-
<i>B. megaterium</i>	10µl	-	-	-	-	-	-
	20µl	-	-	-	-	-	-
	30µl	10	-	-	8	-	-

“-”: no activity observed.

Table 3. MIC and MBC/MFC results (Active concentration - mg.mL⁻¹)

	<i>Bryum capillare</i> var. <i>capillare</i>		<i>Plagionnium affine</i>		<i>Homalothecium sericeum</i>		<i>Homalia besseri</i>		<i>Amblystegium tenax</i>		<i>Homalothecium lutescens</i>	
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
<i>C. albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. enterica</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. flexneri</i>	20	80	-	-	10	80	-	-	40	80	-	-
<i>B. megaterium</i>	20	40	-	-	-	-	20	80	-	-	-	-

“- “:no activity observed.

ÖZET: Karayosunlarının yaraların işleşmesinde etkili olduğu yüzyıllardan beridir bilinmektedir. Daha önce yapılmış olan çalışmalar, karayosunlarına ait bazı sekonder metabolitlerin bazı mikroorganizmalara karşı anti-infektif etki gösterdiğini göstermektedir. Bu çalışmada, Kastamonu yöresinden toplanan *Bryum capillare* var. *capillare*, *Plagiomnium affine*, *Homalothecium sericeum*, *Homalia besseri*, *Amblystegium tenax* ve *Homalothecium lutescens* örneklerinin, *Candida albicans* ATCC 26555, *Salmonella enterica* Serotype Typhium SL 1344, *Escherichia coli* ETEC LM 63083, *Shigella flexneri* ve *Bacillus megaterium* üzerine *in vitro* etkileri çalışılmıştır. Örneklerin antimikrobiyal etkileri disk difüzyon metodu ile ölçülmüş olup, sonuçlar MİK (Minimum inhibisyon konsantrasyonu) ve MBK/MFK (minimum bakterisidal konsantrasyonu/minimum fungisidal konsantrasyonu) testleri ile desteklenmiştir. Sonuçlara göre, karayosunu örneklerinden *Bryum capillare* var. *capillare*, *S. flexneri* ve *B. megaterium* üzerine etki gösterirken; *Homalothecium sericeum* ve *Amblystegium tenax*, sadece *S. flexneri* üzerine, *Homalia besseri* ise sadece *B. megaterium* üzerine antimikrobiyal etki göstermiştir.

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