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Antimicrobial screening of *Mnium stellare*

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Abstract

Many plants contain active substances that are known to be effective in both enhancing the wound healing process and lowering the incidence of wound infections. Previous studies have shown that bryophytes produce a variety of secondary metabolites that present pharmaceutical activities including antimicrobial activity against various pathogenic bacteria and fungi. The aim of this study was to investigate the antimicrobial activity of *Mnium stellare* against 17 bacterial and 1 fungal strains. Our present study has shown that the ethanol extract of *M. stellare* has antimicrobial activity against several Gram positive and negative microorganism tested, but its antimicrobial activity is notable especially against *B. subtilis*, *S. typhimirium*, *S. aureus*, *S. carnosus*, and *S. epidermidis*. These results are the very first report of the antimicrobial activity of *M. stellare*.

Introduction

It has been known for years that in Traditional Chinese Medicine Bryophytes were used to treat several diseases such as cardiovascular diseases, tonsillitis, bronchitis, cystitis and skin infections especially. In addition, previous studies presented that some of the secondary metabolites extracted from Bryophytes are effective in wound healing process and have anti-infective effect on some microorganisms (Altuner et al., 2010).

Especially in last decades the anti-infective activities of plant-derived products come up focus of interest. Antibacterial and antifungal resistance rates of microorganisms accelerated the research on new antimicrobial agents due to increasing morbidity and mortality rates of bacterial and fungal infections (Basile et al., 1998; Ilhan et al., 2006; Agoramoorthy et al., 2007; Veljic et al., 2008; Altuner, 2008; Altuner and Çetin, 2009; Altuner et al., 2010a, 2010b, 2010c, 2011a; Savaroğlu et al., 2011a, 2011b; Onbaşılı et al., 2011, 2013; Oztopcu-Vatan et al., 2011; Savaroglu et al., 2011a, 2011b; Altuner and Canlı, 2012; Altuner et al., 2014).

The secondary metabolites synthesized by plants have several advantages for plants themselves such as acting as a defence mechanism against microorganisms, insects and herbivores (Samidurai and Saravanakumar, 2009, Altuner et al., 2012c). These secondary metabolites have some applications such as being used as antimicrobial agents. 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; Canlı et al, 2014).

In this study, antimicrobial activity of *Mnium stellare* was determined against 17 bacterial and 1 fungal strains.

Materials and Methods

Moss samples

Bryophytes are plants which define about 14,500

species (Veljic et al., 2008). In contrast to the extensive utilisation of higher plants as a source of antimicrobial substances, Bryophytes have rarely been considered for this purpose (Basile et al., 1998). *M. stellare* Hedw. (Family: Mniaceae Schwagr.) samples used in this study were collected from Akdağ Mountain (N 40° 44′ - E 035° 59′), Amasya, which is located bet-ween Central Anatolia and the Middle Black Sea region. *M. stellare* is a well-known species, easily recognised by its lighter green color and unbordered leaves. (Smith, 2004). Voucher specimens were deposited for further reference.

Extraction procedure

All *M. stellare* samples were dried after collection and the samples were ground by a mortar and a pestle. In order to extract active substances, ethanol (Merck, Germany) was chosen as an extraction solvent. Ground samples were shaken in ethanol at 100 rpm for 3 days at room temperature. All the extracts were filtered through Whatman No. 1 filter paper into evaporation flasks (Altuner et al., 2011b). The filtrate was evaporated by a rotary evaporator (Heidolph Hei-Vap Value HL/HB-G1) at 30°C. After evaporation the residues were collected and used to prepare 9 mg/mL extracts.

Microorganisms

A wide range of Gram positive and negative bacteria and yeast were selected to test the antimicrobial effect of *M. stellare*. The strains were chosen from standard strains as much as possible. Other strains which are not standard were all isolated from food and identified in Ankara University, Faculty of Science, Department of Biology.

Bacillus subtilis ATCC 6633, *Candida albicans* ATCC 10231, *Enterobacter aerogenes* ATCC13048, *Enterococcus durans*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Escherichia coli* CFAI, *Klebsiella pneumoniae*, *Listeria monocytogenes* ATCC 7644, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923, *Staphylococcus carnosus* MC1.B, *Staphylococcus epidermidis* DSMZ 20044 and *Streptococcus agalactiae* DSMZ 6784 were used in the study.

Prepartion of inocula

All bacterial strains were incubated at 37°C for 24 hours. But since the requirements for *C. albicans* is different, *C. albicans* was inoculated 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, thus standard inocula is adjusted to contain approximately 10^8 cfu/mL for bacteria and 10^7 cfu/mL for *C. albicans* (Hammer et al., 1999; Altuner, 2011).

Disk diffusion method

Disk diffusion test was performed as described previously by Andrews (2003). The culture medium was poured into 120 mm sterile Petri dish to give a mean depth of 4.0 mm ± 0.5 mm (Altuner and Çetin, 2009; Altuner and Akata, 2010). 60, 100 and 150 µL aliquots of each extract was applied on sterile paper disks of 6 mm diameter end up with 440, 917 and 1375 µg/µL sample on each disk (Mahasneh and El-Oqlah, 1999; Silici and Koc, 2006). To get rid of any residual solvent which might interfere with the results, disks were left to dry overnight at 30°C in sterile conditions (Silici and Koc, 2006; Altuner et al., 2012a, 2012b). 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 min at room temperature before applying the disks. Disks 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.

Controls

Empty sterile disks and extraction solvent (ethanol) loaded on sterile disks which were dried at sterile conditions to remove solvent as done in the study were used as negative controls.

Table I

Disk diffusion test results (Inhibition zones in mm)

	50 µL	100 µL	150 µL
B. subtilis ATCC 6633	8	11	14
C. albicans ATCC 10231	-	-	-
E. aerogenes ATCC13048	-	-	-
E. durans	-	-	-
E. faecalis ATCC 29212	-	-	-
E. faecium	-	-	-
E. coli ATCC 25922	-	-	-
E. coli CFAI	-	-	-
K. pneumoniae	-	-	-
L. monocytogenes ATCC 7644	7	7	9
S. enteritidis ATCC 13075	7	7	8
S. infantis	8	11	12
S. kentucky	8	10	11
S. typhimurium SL 1344	-	8	13
S. aureus ATCC 25923	11	14	14
S. carnosus MC1.B	17	22	24
S. epidermidis DSMZ 20044	20	23	24
S. agalactiae DSMZ 6784	-	-	-

"-": No activity observed

Results

The diameter of the inhibition zones recorded as the diameter of the zones in millimetres for the samples are given in Table I. No activity was observed for the negative controls; solvents and empty sterile disks. Table I clearly shows that ethanol extracts of *M. stellare* were presented antimicrobial activity against *B. subtilis*, *L. monocytogenes*, *S. enteritidis*, *S. infantis*, *S. kentucky*, *S. typhimurium*, *S. aureus*, *S. carnosus* and *S. epidermidis*.

Discussion

Results given in Table I clearly show that *M. stellare* are active against several microorganisms but its antimicrobial activity is notable especially against *B. subtilis*, *S. typhimirium*, *S. aureus*, *S. carnosus* and *S. epidermidis*. There have been no reports about the antimicrobial activity of *M. stellare* as far as the current literature is concerned. These results are the very first data about the antimicrobial activity of *M. stellare*.

Among the microorganisms which were affected by *M. stellare* extracts *B. subtilis*, *L. monocytogenes, S. aureus, S. carnosus and S. epidermidis* are Gram positive where *S. enteritidis, S. infantis, S. kentucky and S. typhimurium* are Gram negative strains.

It is a well known fact that Gram negative bacteria are in general more resistant to a large number of antibiotics and chemotherapeutic agents than Gram positive bacteria (Nikaido, 1998). In addition, it was also pointed out previously that Gram negative bacteria are the dominant killers among bacterial pathogens in the Intensive Care Units (*ICU*) (Villegas and Quinn, 2004).

It was reported that although serovar Typhimurium of *Salmonella* has a less alarming public image than serovar Typhi, it is a bigger health problem and it is thought by researchers to be at least 30-fold underreported. There are probably hundreds of millions of cases every year in the world in which serovar *Typhimurium* kill twice as many people as serovar Typhi which were mostly infants and the elderly people (McClelland et al., 2001). According to results, 100 µL of *M. stellare* extract showed low antibacterial activity against *S. typhimurium*. Since the inhibition zone is quite low, increasing the active substance loaded on the empty sterile antibiotic disks may also increase the activity.

On the other hand ethanol extracts of *M. stellare* are active against several Gram positive strains, as stated previously. The results of the disk diffusion tests applied on the Gram positive strains are more remarkable than the results of Gram negative strains. The activity against especially on *B. subtilis*, *S. carnosus* and *S. epidermidis* are noteworthy.

The pathogenic potential of *B. subtilis* is generally described as low or absent (De Boer and Diderichsen, 1991). *B. subtilis* is only known to cause disease in severely immunocompromised patients (Galieni and Bigazzi, 1998). Several researchers study antimicrobial activity of some plant extracts on *B. subtilis* ATCC 6633. Khalid et al. (2011) compared four different methanolic plant extracts, namely *Pistacia integerrim*, *Polygonum bistorta*, *Swertia chirata* and *Zingiber officinale*. In this study maximum 30 mg of extracts were loaded on sterile antibiotic disks and inhibition zones were found to be 12 mm for *P. integerrim*, 11 mm for *P. bistorta*, 12 mm for *S. chirata* and 17 mm for *Z. officinale*. In our study we observed 14 mm zone for 1.375 mg of *M. stellare* extract which is about 22 times lower than the amount used for study conducted by Khalid et al. (2011). Comparing these results clearly puts forward how *M. stellare* is active against *B. subtilis* when compared to some other higher plants.

S. epidermidis is not usually pathogenic. But they often develop risk for infection for patients with a compromised immune system. These infections can be both nosocomial and community acquired, but they pose a greater threat to patients hospitalized. *S. epidermidis* is also a major concern for people with catheters or other surgical implants because it is known to cause biofilms that grow on these devices (Queck and Otto, 2008; Salyers et al., 2002).

Several studies conducted on the antimicrobial activity of several higher plants against *S. epidermidis*. Mahida and Mohan (2007) tested 10 mg of methanolic extracts of 23 plant extracts, but the highest zone was found to be 20 mm for *Mangifera indica*. In another study ethanol extracts of 23 plants were tested against *S. epidermidis* and the highest inhibition zone diameter was given as 18 mm for *Stachys leptoclada* (Sarac and Ugur, 2007).

In our study we observed a 24 mm inhibition zone for 1.375 mg of *M. stellare* extract against *S. epidermidis* which is relatively high when compared to other previous studies.

Scientists defined *S. kentucky* as a "superbug" since it can develop resistance to some antibiotics, which means it is difficult to treat. It was previously uncommon but after 2006 an increase was observed in *S. kentucky* cases especially in Northeast Africa and Turkey. This strain display high-level resistance to ciprofloxacin, one of the drugs used against *Salmonella* diseases. In addition, secondarily acquired resistances to extended-spectrum cephalosporin and trimethoprim + sulfamethoxazole was also observed (Collard et al., 2007).

As a result, the ethanol extract of *M. stellare* has antimicrobial activity especially against *B. subtilis*, *S. typhimirium*, *S. aureus*, *S. carnosus,* and *S. epidermidis*. But further researches, especially cytotoxicity and genotoxicity tests are needed to be conducted to

conclude whether *M. stellare* extracts can be used safely in terms of their antimicrobial activity.

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