

***In vitro* Antimicrobial Screening of *Hypnum andoi* A.J.E. Sm.**

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Abstract

The tremendous increase in use of the anti-infective drugs is stated as the major factor in microbial evolution and antibiotic resistance. Thus, discovering new alternative antibiotics and antifungals are very important for the future of the public health. The aim of this study is to determine the *in vitro* antimicrobial activity of *Hypnum andoi* A.J.E. Sm. ethanolic extracts against 17 bacterial and 1 fungal strains, namely *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 by using the disc diffusion method. As a result of the study, it is observed that *H. andoi* extracts has antimicrobial activity against several gram positive and gram negative microorganism including *C. albicans*, *E. aerogenes*, *E. coli*, *K. pneumonia*, *S. carnosus* and *S. kentucky*.

Keywords: *Hypnum andoi*, antimicrobial activity, antimicrobial screening, ethanolic extract.

***Hypnum andoi* A.J.E. Sm.'nin *in vitro* Antimikrobiyal Etki Taraması**

Özet

Anti-enfektif ilaçların kullanımındaki büyük artış, mikrobiyal evrim ve antibiyotik direncin ana sebebi olarak gösterilmektedir. Bu sebeple, halk sağlığının geleceği için yeni alternatif antibiyotik ve antifungalların keşfedilmesi büyük önem taşımaktadır. Bu çalışmanın amacı, *Hypnum andoi* A.J.E. Sm. etanol ekstraktlarının *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 ve *Streptococcus agalactiae* DSMZ 6784 olmak üzere 17 bakteri ve 1 mantar suşuna karşı *in vitro* antimikrobiyal etkisinin disk difüzyon yöntemi kullanılarak belirlenmesidir. Sonuç olarak, *H. andoi* ekstraktlarının *C. albicans*, *E. aerogenes*, *E. coli*, *K. pneumonia*, *S. carnosus* ve *S. kentucky* gibi bazı gram pozitif ve gram negatif mikroorganizmaya etki gösterdiği gözlenmiştir.

Anahtar Kelimeler: *Hypnum andoi*, antimikrobiyal aktivite, antimikrobiyal tarama, etanol ekstraktı.

Introduction

There is a tremendous progress in human medicine in the last decades, but due to microbial evolution and antibiotic resistance as a result of the increase in use of anti-infective drugs, bacterial, fungal and viral diseases are still threatening the public health (Cos et al., 2006; URL1(WHO), 2007; Syed et al., 2010).

When extensive drug resistance is taken into account, the importance of the identification of new alternative antimicrobial agents against resistant

microorganism can easily be understood (Okeke et al., 2005; Paudel et al., 2008).

For this reason, scientists have been conducting intensive researches to identify new antimicrobial agents.

Studies show that natural products have a potential especially against infectious diseases and as a result of this humankind has been using these natural products for hundreds of years to treat several diseases caused by bacteria, fungi, viruses and parasites (Jones, 1996; Clardy and Walsh, 2004; Altuner et al., 2010). Today, some of the drug leads, which can be used against

infections, are provided by natural products. It is known that in many developing countries about 80% of the available therapeutic substances are originated from medicinal plants (Baytop 1999, Keleş et al. 2001).

As far as the current literature is concerned, it is obvious that only a very small amount of the available diversity among living organisms have yet been explored for such purposes (Cos et al., 2006).

Hypnum andoi A.J.E. Sm. (Family: Hypnaceae Schimp.) is a common pleurocarpic moss on rocks in many upland districts. Sometimes it forms a cap to large boulders in treeless upland sites, but generally its abundance will decrease away from the shade of woodland. *H. andoi* has characteristic leaves which are strongly curved towards the underside of the shoot and gradually tapering to the apex.

In this study the antimicrobial activity of *H. andoi* is investigated against *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 by using the disc diffusion method.

Materials and Methods

Moss samples

Hypnum andoi A.J.E. Sm. samples used in this study were collected from Akdağ Mountain, Amasya, which is located between Central Anatolia and the Middle Black Sea region. All samples collected were identified by Kerem CANLI. Voucher specimens were deposited for further reference in Herbarium of Ankara University (ANK) Faculty of Science, Department of Biology, Ankara, Turkey.

Extraction procedure

H. andoi samples were air dried after collection and dried samples were grounded by a mortar and a pestle. Ethanol (Merck, Germany) was chosen as the extraction solvent. Grounded samples were shaken in ethanol at 100 rpm for 3 days at room temperature. After 3 days, extracts were filtered through Whatman No. 1 into evaporation flasks. 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⁻¹ extracts.

Microorganisms

Several gram positive and gram negative bacteria and yeast were selected to test the antimicrobial effect of *H. andoi*. Among the selected microorganisms for the study some of them were standard strains where others were isolated from food and identified in Ankara University, Faculty of Science, Department of Biology.

Bacillus subtilis ATCC 6633, *Candida albicans* ATCC 10231, *Enterobacter aerogenes* ATCC 13048, *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.

Preparation of inocula

Bacterial strains were incubated at 37 °C for 24 hours, where *C. albicans* was incubated at 27 °C for 48 hours. Inocula were prepared by transferring microorganisms into 0.9% sterile saline solution. The turbidity of inocula was adjusted according to 0.5 McFarland standard, which yields approximately 10⁸ cfu.mL⁻¹ for bacteria and 10⁷ cfu.mL⁻¹ for *C. albicans* (Hammer et al., 1999).

Disc diffusion method

Disc diffusion test was performed as described previously by Andrews (Andrews, 2003). 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; Altuner and Akata, 2010). 50 μ L, 100 μ L and 150 μ L aliquots of each extract was applied on sterile paper discs of 6 mm diameter end up with 400 μ g. μ L⁻¹, 800 μ g. μ L⁻¹ and 1200 μ g. μ L⁻¹ sample on each disc (Mahasneh and El-Oqlah, 1999; Silici and Koc, 2006). 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; Altuner and Çetin, 2010). The surface of the plates was inoculated by the inocula containing saline suspension of microorganisms. Before applying discs inoculated plates were left to dry for 5 minutes at room temperature. Discs were firmly applied to the surface of the plate. Then plates were incubated and inhibition zone diameters were expressed in millimetres.

Controls

Empty sterile discs and extraction solvent (ethanol) loaded sterile discs were used as negative controls.

Statistics

All extracts were tested in triplicate and *P* values of <0.05 were considered statistically significant.

Results and Discussion

The diameters of the inhibition zones recorded in millimetres are given in Table 1. No activity was observed for the negative controls; extraction solvent and empty sterile discs.

Table 1 clearly puts forward that *H. andoi* samples were presented antimicrobial activity against *C. albicans*, *E. aerogenes*, *E. coli*, *K. pneumonia*, *S. carnosus* and *S. kentucky*.

E. aerogenes, *E. coli*, *K. pneumonia* and *S. kentucky* are gram negative strains. Although the antimicrobial activity against these gram negative strains were very low, these results are very important since 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).

It was reported that antibiotics of natural origin showed >90% lacked activity against *E. coli*, although they were active against gram-positive strains (Vaara, 1993).

It was also previously pointed out that gram negative bacteria are the dominant killers among bacterial pathogens in the Intensive Care Units (*ICU*) (Villegas and Quinn, 2004). One of these gram negative microorganisms is *Klebsiella* which cause death in *ICUs* (Villegas and Quinn, 2004). From this point of view, having antibacterial activity against *E. coli* and *K. pneumoniae* may very important.

One of the “*ICU* bugs” which could cause significant morbidity and mortality was previously identified as *E. aerogenes*. An important point about the infection management of these bacteria is the infection management is complicated due to its resistance to multiple antibiotics (URL2., 2007). Scientists defined *S. kentucky* as a “superbug” since it can develop resistance to some antibiotics, which means it is difficult to treat (URL3, 2011).

It was previously uncommon but after 2006 an increase was observed in *S. kentucky* cases especially in Northeast Africa and Turkey (URL4, 2006). 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)

Although the results against these gram negative strains are low, probably increasing amount of extracts loaded on the empty sterile antibiotic discs may increase the activity.

According to the results it can be concluded that the highest antimicrobial activity observed was against *C. albicans* and *S. carnosus*.

C. albicans is the causal agent of opportunistic oral and genital infections in humans (Ryan and Ray, 2004; Enfert and Hube, 2007). In the study we observed 10 mm of inhibition zone for 1200 μ g. μ L⁻¹

sample loaded on the disc, which can be accepted as notable.

Table 1. Disc diffusion test results (Inhibition zones in mm)

	40µL	60µL	100µL
<i>B. subtilis</i> ATCC 6633	-	-	-
<i>C. albicans</i> ATCC 10231	7	8	10
<i>E. aerogenes</i> ATCC13048	-	-	7
<i>E. durans</i>	-	-	-
<i>E. faecalis</i> ATCC 29212	-	-	-
<i>E. faecium</i>	-	-	-
<i>E. coli</i> ATCC 25922	-	7	7
<i>E. coli</i> CFAI	-	-	-
<i>K. pneumoniae</i>	-	7	7
<i>L. monocytogenes</i> ATCC 7644	-	-	-
<i>S. enteritidis</i> ATCC 13075	-	-	-
<i>S. infantis</i>	-	-	-
<i>S. kentucky</i>	7	7	7
<i>S. typhimurium</i> SL 1344	-	-	-
<i>S. aureus</i> ATCC 25923	-	-	-
<i>S. carnosus</i> MC1.B	8	9	10
<i>S. epidermidis</i> DSMZ 20044	-	-	-
<i>S. agalactiae</i> DSMZ 6784	-	-	-

“-”: No activity observed.

Conclusion

As a conclusion, it can be pointed out that there is an antimicrobial activity of ethanolic extracts of *H. andoi* against *C. albicans*, *E. aerogenes*, *E. coli*, *K. pneumonia*, *S. carnosus* and *S. kentucky*. According to the current literature, there aren't any results reported regarding antimicrobial activity of *H. andoi* until now. These results are the first notable results about the antimicrobial activity of *H. andoi*.

But further researches are needed to be conducted in order to analyse the active substances in details.

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