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Full Length Research Paper

Antifungal and antibacterial effects of some acrocarpic mosses

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In this study, the antifungal and antibacterial effect of 6 different acrocarpous mosses were tested *in vitro* aganist 8 different microorganisms. For the extraction, ethyl alcohol, methyl alcohol, acetone and chloroform were used as solvents. While the highest antimicrobial effect was seen in methyl alcohol extracts, extracts of chloroform showed the lowest level of antimicrobial effect. *Grimmia anodon* Bruch & Schimp. which is one of the acrocarp mosses used in this study, showed the highest activity in terms of the number of microorganism affected. *Tortella tortuosa* (Hedw.) Limpr. only has effect on *Candida albicans* ATCC 16231 strain. All the results were compared with standard antibiotic discs, ketoconazole (50 µg), ampicillin (10 µg), eritromycin (15 µg) and vancomycin (30 µg).

Key words: Moss, acrocarpous, antimicrobial effect.

INTRODUCTION

Bryophytes are the oldest known land plants in the world (Zinsmeister and Mues, 1987). They consist of three separate divisions, the Marchantiophyta (liverworts), Anthocerotophyta (hornworts) and Bryophyta (mosses) (Glime, 2007; Goffinet and Shaw, 2009). Among them, Bryophyta is the largest division of Bryophytes (84% of families and ~98% of species). Based on the morphological characters (branching patterns and location of sexual organs), the Bryophyta has been divided into two major groups as acrocarpous mosses and pleurocarpous mosses. The acrocarpous mosses are generally resistant to drought, by contrast, pleurocarpous mosses are more sensitive to drought (Schofield, 2001).

Many bryophytes exhibit antimicrobial effects against fungi and bacteria (Frahm and Kirchhoff, 2002; Ilhan et al., 2006; Sabovljevic et al., 2006; Basile at al., 1998a, b; 1999; Scher et al., 2004; Subhisha and Subramoniam, 2005; Bodade et al., 2008; Dülger et al., 2009). Almost all species of bryophytes are not damaged by insect larvae, fungi, bacteria, slugs, snails and mammals (Asakawa, 2001) because, biological compounds like oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenylquinone and aromatic and phenolic substances in bryophytes are protected aganist these organisms (Asakawa, 1981, 1984, 1990, 2000). Therefore, bryophytes have the potential for medical use. Traditional medical use of bryophytes in China started more than 400 years ago. For example, *Polytrichum* which is acrocarpous moss and *Fissidens* species were used as diuretic and hair growth stimulating drugs in China (Asakawa, 1990). Moreover, North American Indians used *Bryum, Mnium, Philonotis* and *Polytrichum juniperinum* which are acrocarpous mosses to heal burns, bruises and wounds (Asakawa, 1990; Ilhan et al., 2006).

In this study, we investigated the antifungal and antibacterial effect of 6 different acrocarpous mosses (*Syntrichia ruralis* (Hedw.) F. Weber and D. Mohr, *Grimmia anodon* Bruch and Schimp., *Pleurochaete squorrosa* (Brid.) Lindb., *Bryum capillare* Hedw., *Tortella tortuosa* (Hedw.) Limpr. and *Orthotrichum rupestre* Schleich. ex Schwägr.) which were tested *in vitro* aganist 8 different microorganisms (*Bacillus subtilis* RSKK 244, *Staphylococcus aureus* Koag(+), *Bacillus cereus* 863, *Salmonella* sp. 23.1, *Escherichia coli* ATCC 36218, *Pseudomonas aeroginosa* ATCC 27853 and *Saccharomyces cerevisiae* TP(3-2), *Candida albicans* ATCC 16231). The aim of this study was to determine the antifungal and antibacterial effects of some acrocarpic

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Table 1. Antimicrobial activity of Bryophyte extracts in different solvents.

Moss	Solvent	Inhibition zone (mm)							
		B. subtilis **RSKK 244**	***B. cereus*** **863**	***Salmonella*** **sp. *23.1***	***S. aureus*** **Koag (+)**	***S. cerevisiae*** **TP (3-2)**	***C. albicans*** **ATCC 16231**	***P. aeruginosa*** **ATCC 27853**	***E. coli*** **ATCC 36218**
S. ruralis	EA	-	-	8 ±0.3	-	7 ±0.3	-	-	-
	MA	-	-	9 ±1.0	-	7 ±0.0	-	-	7 ±0.1
	A	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-
G. anodon	EA	7 ±0.3	-	8 ±1.1	8 ±0.2	-	-	6 ±0.0	7 ±0.2
	MA	7 ±0.2	-	8 ±0.0	8 ±0.8	8 ±0.5	-	6 ±0.1	7 ±0.0
	A	6 ±0.0	7 ±0.3	7 ±0.7	7 ±0.3	8 ±0.5	-	-	-
	C	7 ±0.5	7 ±0.0	8 ±0.2	-	9 ±0.5	-	-	-
B. capillare	EA	-	7 ±1.0	8 ±0.0	8 ±0.0	-	-	6 ±0.1	-
	MA	-	8 ±0.5	8 ±0.1	8 ±0.2	8 ±0.5	-	6 ±0.1	-
	A	-	-	7 ±0.1	7 ±0.2	8 ±0.5	-	-	-
	C	-	-	8 ±0.0	-	8 ±0.0	-	-	-
P. squarrosa	EA	-	8 ±1.6	-	-	7 ±0.0	-	-	8 ±1.4
	MA	-	8 ±0.3	-	-	7 ±0.0	-	-	7 ±0.8
	A	-	7 ±0.5	-	-	7 ±0.5	-	-	-
	C	-	8 ±0.0	-	-	-	-	-	-
T. tortuosa	EA	-	-	-	-	-	-	-	-
	MA	-	-	-	-	-	-	-	-
	A	-	-	-	-	-	7 ±0.0	-	-
	C	-	-	-	-	-	-	-	-
O. rupestre	EA	-	-	-	6 ±0.0	7 ±0.5	-	-	7 ±0.5
	MA	-	7 ±1.0	-	-	9 ±0.7	-	-	-
	A	9 ±0.0	-	-	6 ±0.2	-	-	-	-
	C	7 ±0.8	6 ±0.1	-	-	8 ±0.5	-	-	-
Ampicillin		21	23	19	18	-	-	21	22
Eritromycin		28	29	30	31	-	-	29	29
Vancomycin		15	16	16	15	-	-	14	-
Ketoconazole		-	-	-	-	24	21	-	-

Values are mean ± SD of 2 different experiments. EA: ethyl alcohol; MA: methyl alcohol; A: acetone; C: choloroform; -: not detected; SD: standard deviation.

mosses and to make a contribution to the pharmaceutical botany studies to be done in the future in Turkey.

MATERIALS AND METHODS

Plant material

Plant materials of this study were collected from the Melendiz Mountain, Okçular village, (Niğde), Turkey, at an altitude of 1095 m, 37°53'44.73'' N, 34°31'26.21'' E, in March 2010 and Pozən□ı, Şeker Pınarı place (Adana), Turkey, at an altitude of 845 m, 37° 27' 08.20'' N, 34^0 52' 03.60'' E, in May 2010. The specimens were identified by Dr. Tülay Ezer. Specimens are deposited in the Herbarium of the Biology Department, Niğde University, Niğde, Turkey.

Preparation of the extracts

Samples of plant were treated with 0.8% Tween 80 aqueous solution to remove epiphytic hosts found on the plant surface. Then, the samples were washed in tap and distilled water, and dried on filter paper. The samples were extracted with different solvents (ethanol, methanol, acetone and chloroform) for 15 min in a liquefier blender until homogenized. The extracts were centrifuged at 400 rpm and the supernatant was dried at 45°C. The samples were dissolved in 100 mg of the dry residue in 10 ml sterile dimethyl sulfoxide (Basile et al., 1998a).

Determination of antimicrobial activity

The plant extracts were tested for antibacterial and antifungal activities through the disc diffusion method, according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997). Mueller-Hinton Agar (MHA) (LAB M) and Sabouraud Dextrose Agar (SDA), (LAB M) sterilized and cooled to 4 to 50°C, were distributed in sterilized Petri dishes. The filter paper discs (6 mm in diameter, Whatman No:1) were individually impregnated with 20 µl of the extract solutions (filtered with a pore size of 0.45 µm) and then placed onto the agar plates, which had previously been inoculated with tested microorganisms (100 µl). Plates were inoculated with bacteria incubated at 37°C for 24 h and 30°C for 48 h for the yeast strains. The diameter inhibition zones were measured in mm. All the tests were performed in duplicate. Studies performed in duplicate and the inhibition zones were compared with those of reference discs. Reference discs used for control are as follows: Ketoconazole (50 µg), ampicillin (10 µg), eritromycin (15 µg) and vancomycin (30 µg).

RESULTS AND DISCUSSION

In this study, the antimicrobial effects of six plants (*S. ruralis* (Hedw.) F. Weber & D. Mohr, *G. anodon* Bruch & Schimp., *P. squorrosa* (Brid.) Lindb., *B. capillare* Hedw., *T. tortuosa* (Hedw.) Limpr. and *O. rupestre* Schleich. ex Schwägr), having four different solvent extract (ethanol, methanol, acetone and chloroform), were compared with standard antibiotics used as positive controls. Antimicrobial activity of plant extracts in different solvents on test microorganisms are given in Table 1.

The results indicated that ethanolic and methanolic extracts of *S. ruralis* had inhibition effect against *Salmonella, E. coli* and *S. cerevisiae*, while acetone and chloroform extracts were inactive against all test microorganisms. The extract of *B. capillare* was active against only five strains (*B. cereus*, *Salmonella*, *S. aureus*, *P. aeruginosa* and *S. cerevisiae*). The extract of *P. squarrosa* showed moderate activity against some strains but, choloroform extract had prohibitive effect against only *B. cereus* strain. The acetone extract of *G. anodon* was active against only *C. albicans* strain. *O. rupestre* extracts were inactive against *Salmonella, P. aeruginosa* and *C. albicans*.

The antimicrobial test results revealed that *G. anodon* extracts had a potential activity against all microorganisms, except *C. albicans*. While the highest antimicrobial effect was shown in methanol extracts, extracts of chloroform showed the lowest level of antimicrobial effect. The results showed that *S. cerevisiae, Salmonella* and *B. cereus* were found to be more sensitive than the studied test strains (*S. aureus*, *B. subtilis* and *E. coli*). We know that conventional antibiotics are generally more active against the gram positive bacteria than gram negative bacteria. However, these bryophyte samples showed inhibition effect against both the gram positive and negative bacteria. Our results revealed that the selected bryophytes might possess a novel antimicrobial agents. Also, some researchers reported special antimicrobial activities of different bryophyte samples against gram-negative bacteria (Basile et al., 1998a, b; İlhan et al., 2006; Bodade et al., 2008).

On the other hand, our study showed that selected bryophyte samples in our study have antifungal activity against two selected fungi. While *S. cerevisiae* was sensitive against the five plant extracts, this strain was resistance aganist *T. tortuosa* extracts. However, acetone extract of *T. tortuosa* was only active against the *C. albicans*. The results obtained are similar to some researchers report that extracts from mosses displayed antifungal activities (Castaldo-Cobianchi et al., 1988; Bodade et al., 2008).

This study help in the discovery of new antibiotics that could serve as selective agents against infectious diseases.

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