African Journal of Biotechnology Vol. 10(60), pp. 12905-12908, 5 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1126
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Investigation of antimicrobial activity of some Turkish pleurocarpic mosses

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Accepted 21 July, 2011

In this study, the antimicrobial activities of different extracts from the five pleurocarpic mosses (*Platyhypnidium riparioides* (Hedw.) Dixon, *Leucodon sciuroides* (Hedw.) Schwägr., *Hypnum cupressiforme* Hedw., *Homalothecium sericeum* (Hedw.) Br.Eur., and *Anomodon viticulosus* (Hedw.) Hook & Taylor.) were tested aganist eight bacterial and fungal strains. For the extraction, four different solvents (ethyl alcohol, methyl alcohol, chloroform and acetone) were used. While methanolic extracts of *P. riparioides* showed the highest antibacterial effect against the Gram-negative bacterium *Pseudomonas aeroginosa* ATCC 27853, acetone extract of *A. viticulosus* showed the highest antifungal effect against the fungus *Saccharomyces cerevisiae* ATCC. All the results were compared with standard antibiotic discs: ketoconazole (50 µg), amphicillin (10 µg), eritromycin (15 µg), penicillin (10 µg) and vancomycin (30 µg).

Key words: Moss, pleurocarpic, antimicrobial activity.

INTRODUCTION

For a long time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in the world. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). Secondary metabolites which are derived from plants have long been use as drugs and there is an increasing demand for these natural products (Basile et al., 1998a).

Mosses have been used in a lot of areas, on the basis of different features (Altuner, 2008). There are many documents about the use of bryophtes as medicinal plants in China, Europe and North America. For example, some *Fissidens* and *Polytrichum* species were used for diuretic and hair growth stimulating drugs more than 400 years ago in China (Basile et al., 1998b). In addition, 40 kinds of the traditional medicine have been used for cardiovascular system, tonsillitis, bronchitis, tympanitis, cystitis, as well as skin diseases and burns in China (Dülger et al., 2009). Richardson (1981) noted that microorganisms do not play important role in the decay of many moss species. This is becaues antibiotics are produced by mosses (Altuner, 2008). Terpenes, bibenzyls, bisbibenzyls, derivatives of fatty acids and acetophenones are known bryophytes antifungal compounds (Scher et al., 2004) and isoflavonoids, flavonoids and bioflavonoids are reported to be possible chemical barriers against microorganisms (İlhan et al., 2006). Also, *Atrichum, Dicranum, Mnium, Polytrichum* and *Sphagnum* genera's active antibiotic substances have been identified with polyphenolic compounds (Basile et al., 1998c).

Microorganisms have gained resistance to natural and synthetic antibiotics which are used to combat infectious diseases. Due to the increase in side effects, scientist have turned to nature for the discovery of new antimicrobial

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substances. Accordingly, in the treatment of various diseases, the use of drugs derived from plants of natural origin, have increased drastically (Yiğit et al., 2009). There is also evidence in the literature which confirms antibiotic activity of bryophytes against fungi and procaryotic cells. Therefore, in this study, the antimicrobial effects against microorganisms of some pleurocarpic mosses was investigated.

MATERIALS AND METHODS

Plant materials of this study were collected from the Zonguldak Province, at an altitude of ~350 m, 41°24'09.19'' N, 31°49'30.63'' E, in May 2010 (*Leucodon sciuroides*, *Hypnum cupressiforme* and *Anomodon viticulosus*) and Pozanti County (Adana), at an altitude of ~850 m, 37°28'18.53'' N, 34°51'42.44'' E (*Platyhypnidium riparioides*), ~1600 m, 37°25'38.45'' N, 34°55'29.89'' E (*Homalothecium sericeum*) in April 2010. The specimens were identified by Dr. Recep KARA. The specimens are deposited in the herbarium of the Biology Department, Niğde University (Niğde, Turkey) for identification.

Preparation of the extracts

Pleurocarpous moss materials were washed under running tap and distilled water, and dried on filter paper at room temperature to clean off attached litter and dead materials. Then samples of plant were treated with 0.8% Tween 80 aqueous solution to remove epiphytic hosts found on the plant surface (2 to 5 min). The moss samples were extracted with different solvents (ethanol, methanol, acetone and chloroform) for 15 min in a liquefier blender until homogenized. The obtained 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 (DMSO) (Basile et al., 1998a).

Determination of antimicrobial activity

In this study, moss extracts were tested for antimicrobial activity through the disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI, 2005). Mueller-Hinton agar (MHA) (LAB M) and Sabouraud dextrose agar (SDA), LAB M sterilized and cooled to 45 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 micro-organisms (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 millimeters. All the tests were performed in duplicate. Studies were performed in duplicate and the inhibition zones were compared with those of reference discs. Reference discs used for control were as follows: Ketoconazole (50 µg), ampicillin (10 µg), eritromycin (15 µg) and vancomycin (30 µg).

RESULTS AND DISCUSSION

In this study, five pleurocarpic moss species (*P. Ripariodes* (Hedw.) Dixon, *L. Sciuroides* (Hedw.) Schwägr., *H. cupressiforme* Hedw., *H. sericeum* (Hedw.) Schimp, and *A. viticulosus* (Hedw.) Hook &Taylor.), having four different solvent extract (ethanol, methanol, acetonea and chloroform) were tested for antimicrobial activity against six bacterial and two fungal strains. Results were compared with standard antibiotics used as positive controls (Table 1).

The results of the test showed that while methanol extracts of *P. riparioides* showed the highest antimicrobial effect against *Pseudomonas aeruginosa* (8.5 mm), ethanol extracts of *P. riparioides* showed the lowest level of antimicrobial effect against *Saccharomyces cerevisiae* (6.3 mm). All extracts of *P. riparioides* were inactive against only *Candida albicans* (Table 1).

The methanol extract of *L. sciuroides* showed the highest antimicrobial effect with 9.1 mm inhibition zone against only strain (*S. cerevisiae*). The ethanol extract of *L. sciuroides* was active agaist *C. albicans* strain (6.5 mm). But, all extracts of *L. sciuroides* were inactive against only *B. cereus* (Table 1). While chloroform extracts of *H. cupressiforme* showed the highest antimicrobial effect with 8.4 mm inhibition zone against *B. cereus*, methanol extracts showed the lowest antimicrobial effect against *Salmonella* (6.8 mm). All extracts of *H. cupressiforme* were inactive against *Staphyloccocus aureus* (Table 1).

Homalothecium sericeum extracts were inactive against *Salmonella, S. aureus* and *C. albicans*. Acetone extract of *A. viticulosus* had inhibition effect with the highest inhibition zone (11 mm) against *S. cerevisiae*, while all extracts were inactive against *B. subtilis, B. cereus* and *P. aeruginosa* and *Salmonella* sp. (Table 1).

Although, some researchers reported that ethanol, acetone and chloroform extracts of bryophytes were found to be more effective than methanol extract (Bodade et al., 2008; Russell, 2010), methanol extracts of selected moss specimens were quite effective against microorganisms in this study. Methanol extracts of *P. riparioides* showed the highest antimicrobial effect against *P. aeruginosa.*

The antibacterial and antifungal test results in this study revealed that all moss specimens (except for *A. viticulosus*) showed an inhibition effect against the Gram-negative bacteria *P. aeruginosa* and *E. coli*. Chloroform and methanol extracts of *A. viticulosus* were active against only Gram-negative bacterium *Escherichia coli.* It is known that the majority of pathogenic bacteria are Gram-negative and conventional antibiotics are generally more active against the Gram positive bacteria than Gram negative bacteria (Elibol et al., 2011). Besides, some researchers reported antimicrobial activities of different bryophyte samples against Gram negative bacteria (Basile et al., 1998a, b; Ilhan et al., 2006; Bodade et al., 2008). Furthermore, the results of this study showed that all moss specimens had an inhibition effect against the Gram positive bacteria as well as the Gram-negative bacteria (Table 1). Therefore, the selected moss

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

Moss	Solvent	Inhibition zone (mm)							
		***B. subtilis* RSKK 244**	***B. cereus* 863**	***Salmonella* sp. 213**	***S. aureus* Koag (+)**	***S. cerevisiae* TP (3-2)**	***C. albicans* ATCC 16231**	***P. aeruginosa* ATCC 27853**	***E. coli* ATCC 35218**
A. viticulosus	EA	-	-	-	-	8.7±1.1	-	-	-
	MA	-	-	-	6.5±0.1	8.3±1.4	6.9±0.1	-	7.8±0.4
	A	-	-	-	-	11±1.9	-	-	-
	C	-	-	-	7.2±0.2	8.3±0.6	6.5±0.3	-	7.1±0.2
H. sericeum	EA	6.7±0.1	-	-	7.3±0.1	6.5±0.0	-	-	6.5±0.1
	MA	6.4±0.0	6.7±0.2	-	-	7±0.0	-	7.1±0.1	7.4±0.3
	A	7.8±0.2	-	-	8±0.0	6.9±0.5	-	8.3±1.3	8.0±0.3
	C	7.1±0.0	7.1±0.3	-	-	6.8±0.0	-	-	8.2±0.1
H. cupressiforme	EA	-	-	-	-	-	-	-	6.9±0.7
	MA	-	7.3±0.9	6.8±0.6	-	7.7±0.3	-	-	-
	A	-	-	-	-	-	7.0±0.7	-	-
	C	7.6±0.4	8.4±1.2	7.2±1.0	-	7.8±0.1	-	8.0±0.0	-
L. sciuroides	EA	-	-	7.9±0.1	6.6±0.1	7.5±0.2	6.5±0.2	6.5±0.3	-
	MA	-	-	-	-	9.1±0.7	-	-	-
	A	-	-	-	7.6±0.0	7.0±0.2	7.9±0.1	7.8±0.2	-
	C	7.3±0.1	-	-	-	7.3±0.5	-	6.9±0.3	6.7±0.5
P. riparioides	EA	7.0±0.0	6.4±0.1	6.6±0.4	-	6.3±0.1	-	-	-
	MA	6.7±0.2	6.4±0.2	8.0±0.1	7.1±0.4	7.2±0.6	-	8.5±0.5	7.4±0.6
	A	6.4±0.0	7.0±0.5	7.5±0.5	-	-	-	7.3±0.1	7.5±0.1
	C	6.4±0.2	-	7.0±0.0	-	6.6±0.0	-	6.8±0.0	7.0±0.0
Antibiotic									
Amphicillin		21.3	22.8	19.2	18.7	-	-	21.1	22
Eritromycin		28.3	29	30.4	31.7	-	-	29.3	29.4
Penicillin		30.6	30.8	31.3	26.5	-	-	33.1	32.4
Vancomycin		15.1	16.5	16.3	15	-	-	15	-
Ketoconazole		-	-	-	-	24	21	-	-

Values are mean ± SD of 2 different experiments. EA: Ethyl alcohol, MA: methyl alcohol, A: acetone, C: chloroform, not detected.

specimens may be advantageous as antimicrobial agents and they can help for the discovery of new antibiotics.

In addition, this study showed that selected pleurocarpic moss specimens have an antifungal activity aganist two selected fungus (*S. cerevisiae* and *C. albicans*). While *S. cerevisiae* was more sensitive against the five plant extracts, *C. albicans* showed more resistance aganist extracts of *P. riparioides* and *H. sericeum*. The results obtained are similar to the results of some researchers who reported that extracts from mosses displayed antifungal activities (Castaldo et al., 1988; Bodade et al., 2008; Elibol et al., 2011).

This study will help for the discovery of new wide-spectrum antibiotics that can serve as selective agents against infectious diseases.

ACKNOWLEDGEMENT

The authors are indebted to the Research Fund of Niğde University for financial support (Project no: FEB2010/35).

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