Full Length Research Paper

Investigation of antimicrobial activity of some Turkish pleurocarpic mosses

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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 against 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), ampicillin (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 bryophytes 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 because 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...
substances. Accordingly, in the treatment of various diseases, the use of drugs derived from plants of natural origin, have increased drastically (Yigit et al., 2009). There is also evidence in the literature which confirms antibiotic activity of bryophytes against fungi and procar-yotic 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 lequier 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 microorganisms (100 µl). Plates were 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. riparioides (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, acetone 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 against 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 Staphylococcus 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 (Elbıl 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.

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

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 against two selected fungi (S. cerevisiae and C. albicans). While S. cerevisiae was more sensitive against the five plant extracts, C. albicans showed more resistance against 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.

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REFERENCES


