Asian Journal of Chemistry Vol. 21, No. 5 (2009), 4093-4096

Evaluation of Antimicrobial Activity of Some Mosses From Turkey

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Ethanolic extracts obtained from 5 Byrophyte species (*Platyhypnidium riparioides* (Hedw.) Dixon, *Anomodon viticulosis* (Hedw.) Hook & Taylor, *Polytrichostrum formosum* (Hedw.) G.L.Sm., *Plasteurhynchium meridionale* (Schimp.) M. Fleish. and *Ctenidium molluscum* (Hedw.) Mitt. were tested against *Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas putida, Salmonella typhimurium, Salmonella typhi, Neisseria gonorhoeae, Candida albicans, Rhodotorula rubra, Kluyveromyces fragilis, Kluyveromyces marxianus* and *Debaryomyces hansenii* by disc diffusion method. The extracts of the plants especially *P. meridionale* and *A. viticulosis* were active on all tested microorganisms.

Key Words: Antimicrobial activity, Mosses, Plant extract.

INTRODUCTION

Bryophyte species began to be used as medicinal plants more than 400 years ago in China, Europe and North America[1]. Burned ash of mosses mixed fat and honey is used as ointment for cuts, burns and wounds in the Himalayan region. In addition, Chinese traditional medicine names 40 kinds of the cardiovascular system, tonsillitis, bronchitis, tympanitis, cystitis, as well as skin diseases and burns[2]. A few moss genera like *Atrichum, Dicranum, Mnium, Polytrichum* and *Sphagnum* possess antibiotically active substances[1-4]. Antibacterial, antifungal and antiviral activity are also known for several Bryophyte species extracts[1,5-9].

In this work, the ethanolic extracts obtained from wild-growing *Platyhypnidium riparioides* (Hedw.) Dixon, *Anomodon viticulosis* (Hedw.) Hook & Taylor, *Polytrichostrum formosum* (Hedw.) G.L.Sm., *Plasteurhynchium meridionale* (Schimp.) M. Fleish. and *Ctenidium molluscum* (Hedw.) Mitt. in Turkey have been investigated for their antimicrobial activity.

EXPERIMENTAL

The plant materials were collected from different localities of Turkey in May 2008. Voucher specimens have been deposited in Herbarium of Canakkale Onsekiz Mart University, Turkey.

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Preparation of the extracts: Samples of the plants were treated with 0.8 % Triton-X-100 in water solution to remove epiphytic hosts normally found on the surface, extensively washed in tap and distilled water and dried on filter paper. Unknown parts of the plant were removed during the washing process. The plant material (10 g) was dried in the open air at room temperature. This was then finely ground with a hammer mill and extracted separately with 95 % ethanol (200 mL) for 48 h[10]. The extracts were then filtered through a Buchner funnel and the solvent was removed under reduced pressure at 60-65 ºC on a rotary evaporator. The extract was removed and dried completely at 37 ºC, kept at 4 ºC in a dessicator and were tested for antimicrobial activity within 10 d after preparation.

Microorganisms: *Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas putida, Salmonella typhimurium, Salmonella typhi, Neisseria gonorhoeae, Candida albicans, Rhodotorula rubra, Kluyveromyces fragilis, Kluyveromyces marxianus* and *Debaryomyces hansenii* were used as test microorganisms.

Determination of antimicrobial activity: The plant extracts were tested for antimicrobial activity through the disc diffusion method according to the National Committee for Clinical Laboratory Standards[11]. The dried plant extracts were dissolved in 10 % aqueous dimethyl sulfoxide to a final concentration of 200 mg/mL and sterilized by filtration through an 0.45 µm membrane filter. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Schull No:2668, Dassel, Germany) were each impregnated with 50 µL extract (10 mg/disc) at concentration of 200 mg/mL. All the bacteria mentioned above were incubated 35 ± 0.1 ºC for 24 h inoculation into Nutrient Broth (Difco Laboratories, MI, USA) and the yeast cultures studied were incubated in Malt Extract Broth (Difco Laboratories, MI, USA) at 25 ± 0.1 ºC for 48 h an inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate), the discs injected with extracts were placed at 4 ºC for 2 h, plaques injected with the yeast cultures were incubated at 25 ± 0.1 ºC for 72 h and bacteria were incubated at 35 ± 0.1 ºC for 24 h.

At the end of the period, inhibition zones formed on the medium were evaluated appropriate reference antibiotic disc was applied, depending on the test microorganisms for comparison.

RESULTS AND DISCUSSION

Table-1 shows antimicrobial activity of the mosses extracts and the inhibition zones formed by standard antibiotic discs. Inhibition of bacterial growth was compared with that of chloramphenicol, while the antifungal activity was compared with that of clotrimazole. It was found that the ethanolic extracts of the plants have greater antibacterial effect than those of antifungal effects.

TABLE-1
ANTIMICROBIAL ACTIVITY OF THE MOSSES

Microorganisms		Zone of inhibition (mm)[a]					
		EtOH extracts					Standards[b]
		1	2	3	4	5	
Bacteria							Chl
Bacillus cereus	G^+	15.6	11.4	12.2	18.2	11.2	16.0
Bacillus subtilis	G^+	15.2	14.2	14.2	12.0	12.4	16.2
Micrococcus luteus	G^+	14.8	10.9	10.6	10.8	11.6	18.2
Staphylococcus aureus	G^+	15.6	14.8	12.6	22.2	12.6	18.2
Escherichia coli	G^-	15.4	12.8	14.2	12.4	10.8	18.4
Enterobacter aerogenes	G^-	16.2	12.8	14.6	11.8	10.6	18.4
Proteus vulgaris	G^-	16.8	15.8	13.2	15.6	9.8	18.4
Proteus mirabilis	G^-	14.8	18.8	10.6	17.2	9.6	16.6
Pseudomonas aeruginosa	G^-	15.8	14.8	12.4	10.2	10.4	24.8
Pseudomonas putida	G^-	14.8	10.2	10.4	9.6	9.6	20.4
Salmonella typhimurium	G^-	15.8	14.6	14.2	9.4	11.4	16.8
Salmonella typhi	G^-	15.2	13.8	12.2	10.4	10.4	16.6
Neisseria gonorrhoeae	G^-	16.6	12.6	9.2	15.2	10.6	18.2
Fungi							Clo
Candida albicans		14.4	11.2	11.2	16.4	10.8	15.8
Rhodotorula rubra		14.2	12.2	12.6	14.4	12.4	16.4
Kluyveromyces fragilis		15.6	14.6	12.4	14.2	11.8	18.6
Kluyveromyces marxianus		14.8	11.8	11.4	15.3	10.6	16.4
Debaryomyces hansenii		14.4	12.4	12.6	14.8	11.6	20.4

1 = *P. riparioides*, 2 = *A. viticulosus*, 3 = *P. formosum*, 4 = *P. meridionale*, 5 = *C. molluscum*
[a]Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates.
[b]Chl: Chloramphenicol (10 µg/disc) for bacteria; Clo: Clotrimazole (30 µg/disc) for fungi.

When compared to standard antibacterial antibiotic chloramphenicol, the antibacterial activity has moderate against the tested bacteria. Notably, *Staphylococcus aureus* appeared more sensitive to the extract of *P. meridionale*. Similarly, the extracts of *A. viticulosis* and *P. meridionale* have a strong antibacterial activity against *Proteus mirabilis*. The yeast cultures *Candida albicans* showed the greatest sensitivity to the extract of *P. meridionale* among fungi. The extracts of the plants have a moderate activity against the other fungal test organisms.

Of Bryophyte extracts, the simplest land plants, isoflavonoids, flavonoids and bioflavonoids have been reported to be possible chemical barriers against microorganisms[12-16]. Terpenoids, phenolic and volatile constituents have also investigated in some Bryophyte species[17]. It was found that a methanolic extract of *H. aduncus* inhibited the growth of pathogenic fungi *Botrytis cineria*, *Rhizoctonia soloni* and *Pythium debaryanum*, whereas petroleum-ether extracts of *Barbula* and *Timmiella* species were found to be active against both gram-positive and gram- negative bacteria. *Plagiochila stevensoniana* proved to inhibit dermatophytic organisms like *Trichophyton mentagrophytes*, *Candida albicans* and *Bacillus subtilis*. This antibiotic

activity might be attributed due to the presence of non-ionized organic acids and polyphenolic compounds. In case of several Bryophyte species, the active ingredients responsible for antimicrobial effects have been identified, *e.g.*, Polygoclial from *Porella*, Norpiguisone from *Conocephalum conicum* and Lunularin from *Lunaria cruciata*[18]. Ethanolic extract has antimicrobial activity against both gram-positive and gram negative bacteria as well as fungi used in this study. The results are similar to those reported above. There is no literature data available on the phytochemistry of the Bryophyte species used in this study. The mentioned substances may be responsible for antimicrobial activity. Besides, ethanol was observed as the best solvent for extracting antimicrobial substances in a previous study[19]. The results in this study with ethanol are similar to those reported in that study. It is important to bear in mind that the concentration of extract used in the test may be correlated with a high activity of its chemical components.

In conclusion, the extracts demonstrating especially antimicrobial activity against *Staphylococcus aureus*, *Proteus mirabilis* and *Candida albicans* could result in the discovery of novel antimicrobial agents, showing broad spectrum activities. This may help to discover new antibiotics that could serve as selective agents against infectious diseases.

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(*Received*: 24 November 2008; *Accepted*: 25 February 2009) AJC-7291