Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

In vitro Antimycobacterial Potential of Some Fresh-water Macroalgae and Aqueous Plants

I. Orhan, B. Sener, T. Atici & P. Palittapongarnpim

To cite this article: I. Orhan, B. Sener, T. Atici & P. Palittapongarnpim (2002) In vitro Antimycobacterial Potential of Some Fresh-water Macroalgae and Aqueous Plants, Pharmaceutical Biology, 40:8, 568-569, DOI: 10.1076/phbi.40.8.568.14651

To link to this article: https://doi.org/10.1076/phbi.40.8.568.14651

Pharmaceutical Biology
2002, Vol. 40, No. 8, pp. 568–569

1388-0209/02/4008-568$16.00

In vitro Antimycobacterial Potential of Some Fresh-water Macroalgae and Aqueous Plants

I. Orhan[1], B. Şener[1], T. Atici[2] and P. Palittapongarnpim[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey; [2]Department of Biology, Faculty of Education, Gazi University, Ankara, Turkey, and [3]Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand

Abstract

Crude extracts prepared from fresh-water macroalgae, *Cladophora fracta* (Dilw.) Kütz, *C. glomerata* (Dilw.) Kütz, *Spyrogyra gratiana* Link., and *Maugeotia* sp. (C.A. Agardh) Wittrock, along with two aqueous plants, *Elodea canadensis* Michx. and *Ranunculus rionii* Lagger have been investigated for their antimycobacterial activity. The results of the crude extracts are described in this study.

Keywords: Antimycobacterial activity, tuberculosis, fresh-water macroalga, aqueous plant, *Mycobacterium tuberculosis*.

Introduction

Tuberculosis (TB), a chronic bacterial infection, causes more deaths worldwide than any other infectious disease. In people with weakened immune systems, especially those infected with the human immunodeficiency virus (HIV), TB organisms may overcome the body's defenses, multiply, and cause active disease. TB kills about 2 million people each year (Dormandy, 1999).

With appropriate antibiotic therapy, TB can usually be cured. In recent years, however, drug-resistant cases of TB have increased dramatically. Particularly alarming is the increase in the number of people with multi-drug resistant TB (MDR-TB), caused by *Mycobacterium tuberculosis* strains resistant to two or more drugs. Even with treatment, the death rate for MDR-TB patients is 40 to 60%. The most common drugs, also called first line drugs, are isoniazid (INH), rifampin, pyrazinamide, ethambutol, and streptomycin. Treatment for MDR-TB often requires the use of a second line of TB drugs, such as ethionamide, cycloserine, capreomycin, amikacyn, kanamycin, thiocetazone, quinolones (ofloxacin, ciprofloxacin, sparfloxacin) and macrolides (clarithromycin, clofazimine, amoxycillin and clavulonic acid), all of which have serious side effects (Breathrach et al., 1998; Crofton et al., 1999).

Against this depressing background of the disease and drug resistance increase, new approaches and drugs must be developed in the battle against tuberculosis. Aqueous weeds and algae have been confirmed to be the rich sources of bioactive compounds with desirable activities such as antitumor, antiviral and antiparasitic (Schmitz et al., 1992). As a part of our ongoing research on screening of biologically active substances from aqueous organisms collected from Turkish waters, we have investigated antimycobacterial activity of four fresh-water macroalgae, *Cladophora fracta* (Dilw.) Kütz, *C. glomerata* (Dilw.) Kütz, *Spyrogyra gratiana* Link. and *Maugeotia* sp. (C.A. Agardh) Wittrock, along with two aqueous plants, *Elodea canadensis* Michx. and *Ranunculus rionii* Lagger, against *Mycobacterium tuberculosis* strain H37Ra, using the microplate Alamar blue assay (MABA).

Materials and method

Plant collections

Collection sites and dates of fresh-water macroalgae, *Cladophora fracta* (Dilw.) Kütz, *C. glomerata* (Dilw.) Kütz, *Spyrogyra gratiana* Link., *Maugeotia* sp. (C.A. Agardh) Wittrock, in addition to two aqueous plants, *Elodea canadensis* Michx. and *Ranunculus rionii* Lagger, are listed in Table 1. Voucher specimens are preserved in the Herbarium of Faculty of Pharmacy of Gazi University, Ankara.

Accepted: April 15, 2002

Address correspondence to: I. Orhan, Dept. of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey.

Table 1. Collection sites, dates and herbarium numbers of the mentioned plants.

Plant Species	Collection Site	Collection Date	Herbarium No
Cladophora fracta	Beyşehir Lake, Konya	May, 1999	GUE 2179
C. glomerata	Ceyhan River, Elbistan	April, 1999	GUE 2178
Spyrogyra gratiana	Mogan Lake, Ankara	April, 1999	GUE 2180
Maugeotia sp.	Sariyer Damn, Kirikkale	May, 1999	GUE 2181
Elodea canadensis	Ceyhan River, Elbistan	April, 1999	GUE 1606
Ranunculus rionii	Beyşehir Lake, Konya	May, 1999	GUE 1510

Table 2. Antimycobacterial activity results of fresh-water macroalgae and plants against *Mycobacterium tuberculosis* H37Ra.

Plant species	Results against TB at 200 µg/ml	MIC values (µg/ml)
Cladophora fracta	Active	50
Cladophora glomerata	Inactive	–
Spyrogyra gratiana	Inactive	–
Maugeotia sp.	Active	200
Elodea canadensis	Inactive	–
Ranunculus rionii	Inactive	–

Preparation of plant extracts

Plant materials were cleaned, washed and air-dried. Powdered samples (10 g) were weighed accurately, macerated in ethanol (80%, 50 ml) at room temperature twice and filtered through filter paper. The filters were rinsed with another 50 ml of ethanol and the combined filtrates were evaporated to dryness in *vacuo*. Equal amounts (approximately 25 mg) of each extract were weighed and used for antituberculosis activity tests.

Antimycobacterial assay

The method used in antimycobacterial activity assay was microplate Alamar blue assay (MABA) (Collins & Franzblau, 1997). Suspensions of *Mycobacterium tuberculosis* H37Ra strains were prepared at about 10^5 cells/ml. The bacterial suspension (100 µl) was added to each well of a microtiter plate together with the plant extracts in Middlebrook 7H9 medium to a final volume of 200 ml and the final concentration of the plant extracts at 50, 100 and 200 µg/ml. Following incubation for about 7 days, 20 µl of Alamar blue dye was added to the control well. If the dye turned pink, indicating bacterial growth, the dye was then added to all remaining wells in the plate. The results were read the following day. If the extracts were active at 50 µg/ml, MIC values of the extracts were calculated. As to the standard of tests, MIC values of rifampin, isoniazid and kanamycin were determined for each batch of testing. The acceptable MIC ranges of the drugs were between 0.0047–0.0095, 0.05–0.1 and 2.5–5.0 µg/ml, respectively.

Results and discussion

The results of antimycobacterial activity assay of the crude extracts are summarized in Table 2. In the assay, the change in color of Alamar blue from blue, in the oxidized form, to pink, in the reduced form, is the indicator of bacterial growth. The reduction reflects consumption of oxygen, which is essential for the growth of the obligate-aerobic bacterium. The change in color was observed visually. Active extracts possessing varying degrees of inhibition in the *in vitro* primary screening test at 200 µg/ml concentration were retested at lower concentration to determine the actual minimum inhibitory concentration (MIC).

The extracts of *C. fracta* and *Maugeotia* sp. showed activity at 50 and 200 µg/ml, respectively. Remaining extracts prepared from *C. glomerata*, *S. gratiana*, *E. canadensis* and *R. rionii* were found to be inactive in the test. In qualitative analysis performed on both of the active extracts, the extracts of *C. fracta* and *Maugeotia* sp. gave positive results depending on phenolic and nonpolar compounds. Therefore, it can be stated that both types of compounds in these extracts could be responsible for antimycobacterial activity and further study on the isolation of active compounds from these extracts is in process. To our knowledge, this is the first study on antimycobacterial activity of the aforementioned fresh-water macroalgae and plants.

References

Schmitz F, Bowden BF, Toth SI (1992): Antitumor and cytotoxic compounds from marine organisms. In: *Marine Biotechnology*. Attaway DH, Zaborsky OR, eds, Plenum Press, New York, pp. 197–308.

Breathrach AS, de Reiter A (1998): An outbreak of multi-drug resistant tuberculosis in a London teaching hospital. *J Hosp Infect 2239*: 111–117.

Collins L, Franzblau SG (1997): Microplate Alamar blue assay versus BACTEC460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob Agents Chemother 41*: 1004–1009.

Crofton J, Horne N, Miller F (1999): *Clinical Tuberculosis*. 2nd Edition, Macmillan Press, London.

Dormandy T (1999): *The White Death. A History of Tuberculosis*. Hambildon Press, London.