

Antiviral and cytotoxic activity of some lichen extracts

Ali KARAGÖZ^{1*} & Ali ASLAN²

¹Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, TR-34459 Vezneciler-Istanbul, Turkey; phone: ++ 90 212 455 57 00 /Ext. 15119, fax: ++ 90 212 514 03 66, e-mail: sanicula@istanbul.edu.tr

²Atatürk University, Kazım Karabekir Education Faculty, Biology Department, Erzurum, Turkey

Abstract: The aqueous extracts (AEs) and the ethanolic extracts (EEs) prepared from some lichen species were evaluated for antiviral activity against human parainfluenza virus type 2 (HPIV-2) and cytotoxic activity towards Vero cells. MTT (tetrazolium blue) assay was used to evaluate the reduction of viability of cell cultures in presence and absence of the extracts and plaque assay was used to evaluate the antiviral activity. The AE of *Xanthoria parietina* (L.) Th.Fr. and the EE of *Xanthoparmelia tinctoria* (Maheu & A. Gillet) exhibited attractive antiviral activities. The 50% effective concentration (EC₅₀) of the AE of *X. parietina* for HPIV-2 replication was $10 \pm 6.3 \mu\text{g/mL}$, and the antiviral index (AI) was 50. The EC₅₀ of the EE of *X. tinctoria* for HPIV-2 replication was $20 \pm 2.8 \mu\text{g/mL}$, and AI was 22.5. In an effort to analyze further the mechanism of antiviral activity, the effectiveness of the AE of *X. parietina* and the EE of *X. tinctoria* on different steps of virus replication was examined. The antiviral activities of extracts could be attributed neither to the direct inactivation of the HPIV-2 nor to the inhibition of adsorption to Vero cells. These species are good candidates for further activity-monitored fractionation to identify active principles.

Key words: Lichens, antiviral activity, cytotoxic activity, MTT assay.

Abbreviations: AE, aqueous extract; AI, antiviral index; CC50, 50% cytotoxic concentration; CE50, 50% effective concentration; DMSO, dimethyl sulfoxide; EE, ethanolic extract; EMEM, Eagle's minimum essential medium; EMEM2, EMEM containing 2% fetal calf serum; HPIV-2, human parainfluenza virus type 2; MTT, tetrazolium blue; p.f.u., plaque forming units; PBS, phosphate buffered saline.

Introduction

The lichens are symbiotic organisms of fungi (mycobionts) and algae (phycobionts) comprising about 17,000 species (HUNECK, 1999). Lichens have been used in folk medicine in many countries over a considerable period of time. Lichens, together with some marine organisms and frog venom, are one of the most important sources of biologically active compounds other than plants (BARNES, 2000). They have been used by humans for centuries as food in periods of famine (i.e. during the Leningrad siege), as a source of dye (from the early 1300s), and for their therapeutic properties in traditional medicine. Their efficacy is due to the synthesis of unique secondary compounds, a number of which have important biological roles (PERRY et al., 1999). Lichens synthesize numerous metabolites, the "lichen substances", which comprise amino acid derivatives, sugar alcohols, aliphatic acids, macrocyclic lactones, monocyclic aromatic compounds, quinones, chromones, xanthenes, dibenzofuranes, depsides, depsidones, depsones, terpenoids, steroids, carotenoids and diphenyl

ethers (CLIX et al., 1984; FIEDLER et al., 1986). Lichens and their metabolites have manifold biological activities: antiviral, antibiotic, antitumor, allergenic, plant growth inhibitory, antiherbivore, ecological roles and enzyme inhibitory (DÜLGER et al., 1997; 1998; HUNECK, 1999; ASLAN et al., 1999; 2001).

Host preference and performance of lichenivorous *Eilema* spp. larvae have been related to lichen secondary metabolites (PÖYKKÖ & HYVÄRINEN, 2003). Secondary metabolites of lichens have been found to play important roles for regulating metabolism of lichen thallus alone, e.g. they are sun screening compounds and protecting algal partner against photoinhibition (LEGAZ et al., 1986). Usnic acid, which is a very active lichen substance, is used in pharmaceutical preparations. Usnic acid and vulpunic acid (produced by a mycobiont) are cell division regulators of autotrophic partner of lichen symbiosis-photobiont (BACKOR et al., 1998). Large amounts of *Pseudevernia furfuracea* and *Evernia prunastri* are processed in the perfume industry, and some lichens are sensitive reagents for the evaluation of air pollution. The introduction of new

* Corresponding author