Antimicrobial Activity of Extracts of the Lichen *Parmelia sulcata* and its Salazinic Acid Constituent

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Z. Naturforsch. **62 c**, 619–621 (2007),
received March 8/May 4, 2007

The antimicrobial activity of the acetone, chloroform, diethyl ether, methanol, and petroleum ether extracts of the lichen *Parmelia sulcata* and its salazinic acid constituent have been screened against twenty eight food-borne bacteria and fungi. All of the extracts with the exception of the petroleum ether extract showed antimicrobial activity against *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Proteus vulgaris, Yersinia enterocolitica, Staphylococcus aureus, Streptococcus faecalis, Candida albicans*, *Candida glabrata, Aspergillus niger, Aspergillus fumigatus,* and *Penicillium notatum*. Salazinic acid did not show antimicrobial activity against *L. monocytogenes, P. vulgaris, Y. enterocolitica*, and *S. faecalis* but showed activity against *Pseudomonas aeruginosa* and *Salmonella typhimurium* as well. The MIC values of the extracts and the acid for the bacteria and fungi have also been determined.

Key words: Parmelia sulcata, Salazinic Acid, Antimicrobial Activity

Introduction

Lichens are symbiotic associations between a fungus and an algae (Fahselt, 1994) and about 20000 lichen species are distributed throughout the world. Their chemistry involves the production of a wide range of both primary (intracellular) and secondary (extracellular) compounds (Elix, 1996). Primary metabolites consist of amino acids, polyols, carotenoids, polysaccharides and vitamins. Secondary metabolites that are produced by the fungus partner of the lichen are often called lichen acids and make the majority of organic compounds found in lichens. So far more than 850 secondary metabolites in lichens have been identified, and studies have shown that lichens are forced to produce such compounds as protective substances which are essential for the self-protection of slow growing lichens (Fahselt, 1994; Müller, 2001). That is why many secondary metabolites in lichens are antigrowth, antimicrobial or antiherbivore agents (Huneck, 1999, 2001, 2006; Fahselt, 1994). It appears that more than 50% of the lichen species have substances with antimicrobial activity against human disease organisms. The antibiotic effect of a number of lichen metabolites was generally found to be significant for Gram-positive bacteria, but ineffective against Gram-negative bacteria.

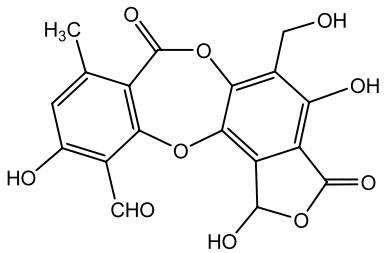

Fig. 1. Chemical structure of salazinic acid.

This study was carried out to screen the antimicrobial activity of extracts of the lichen *Parmelia sulcata* and its salazinic acid constituent. Salazinic acid (Fig. 1) is a β-orcinol-type depsidone (Culberson, 1979). Ingólfsdóttir *et al.* (1998) screened *in vitro* activities of salazinic acid from *Parmelia saxatilis* and four other lichen compounds against *Mycobacterium aurum*. They found a MIC value of 250 mg/ml of salazinic acid against *M. aurum*.

Experimental and Results

Lichen material

Parmelia sulcata Taylor was collected at Bozdağ, Eskişehir Province in Turkey at 1200 m on May 30, 2004. A voucher was stored at the Herbarium of the Department of Biology, Anadolu University (ANES).

Extraction

A air-dried lichen sample of *Parmelia sulcata* was first ground and then 10 g portions were added to 100 ml of the solvents methanol, acetone, diethyl ether, petroleum ether and chloroform. The mixtures were first sonicated for 1 h, then left at room temperature overnight and filtered. The amount of the extract residue in each filtrate was

determined after removal of the solvent of 10 ml aliquots with a rotary evaporator.

Determination of the MIC values of the extracts

Determinations of the MIC values of the extracts of the lichen *Parmelia sulcata* against test bacteria and fungi were carried out according to the Kirby and Bauer disc diffusion method (National Committee for Clinical Laboratory Standards, 1993). The extract residue-containing filter paper discs were prepared the same way as described previously (Yılmaz *et al*., 2004; Tay *et al.*, 2004). From each extract, ten sets of sixty sterilized discs in varying amount of extract residue were prepared. The extract residue contents of the discs varied from 2.56 mg/disc to 5.0 μg/disc for the methanol extract, from 1.28 mg/disc to 2.5 μg/disc for the acetone extract, from 0.21 mg/disc to 0.41 μg/disc for the diethyl ether extract, from 0.48 mg/disc to 0.94 μg/disc for the chloroform extract, and from 0.094 mg/disc to 0.18 μg/disc for the petroleum ether extract.

After inoculation of 250 μl (10^8 cells/ml or spores/ml) solutions of the bacteria onto nutrient agar and of the yeasts and filamentous fungi onto potato dextrose agar, an array of the discs containing different amounts of the extract residue from the same extract was transferred into inoculated microorganism media to determine the MIC value of the extract. Pure solvent-treated and dried discs were used as negative control discs. Chloramphenicol and ketoconazole were used as positive control substances. The bacterial plates were incubated for 24–48 h at 35–37 °C and the fungal plates were incubated for 5 d at 20–25 °C. The MIC values were determined by checking the inhibition zones formed and all MIC value determination experiments were done twice (Tabel I).

Isolation and characterization of salazinic acid and atranorin

According to the bioautographic analysis, two substances in the extracts were antimicrobialy active; they were identified as salazinic acid and atranorin. In order to determine the amounts of salazinic acid and atranorin in each extract, preparative thin layer chromatography (TLC) was employed. After spotting the residues obtained from the methanol, acetone, chloroform, and diethyl ether extracts on different silica gel TLC plates, Silica Gel 60 F254 (20 cm × 20 cm, Merck), the plates were developed in solvent system G which is a mixture of solvents employed in the TLC of lichen substances (Orange *et al.*, 2001; Huneck and Yoshimura, 1996). The solvent system G consists of toluene/ethyl acetate/formic acid

Table I. MIC values of the extracts of *Parmelia sulcata* and its salazinic acid constituent.

Microorganism	MIC (against 10^7 cells or spores)					
	Acetone extract[a] [μg]	Chloroform extract[a] [μg]	Diethyl ether extract[a] [μg]	Methanol extract[a] [μg]	Salazinic acid [μg/41.7 μl]	[mM]
A. hydrophila	80	120	210	40	125	7.7
B. cereus	20	15	26	20	63	3.9
B. subtilis	20	15	53	20	125	7.7
L. monocytogenes	40	60	53	80	–[b]	–
P. vulgaris	160	120	210	640	–	–
Y. enterocolitica	160	60	210	1280	–	–
S. aureus	40	30	26	80	125	7.7
S. faecalis	40	15	26	40	–	–
P. aeruginosa	–	–	–	–	250	15.4
S. typhimurium	–	–	–	–	250	15.4
C. albicans	160	60	210	320	500	30.8
C. glabrata	80	60	210	160	500	30.8
A. niger	640	240	105	320	250	15.4
A. fumigatus	1280	120	105	320	250	15.4
P. notatum	1280	480	210	640	500	30.8

[a] The concentrations of the stock solutions were 3 mg/ml for the acetone extract, 1.12 mg/ml for the chloroform extract, 0.5 mg/ml for the diethyl ether extract, and 6 mg/ml for the methanol extract.
[b] Inactive.

(139:83:8 v/v/v). The spots belonging to salazinic acid and atranorin on each developed plate were located using the R_f values of these compounds given in the literature for the solvent system G (Huneck and Yoshimura, 1996; Orange *et al.*, 2001). Further characterizations of salazinic acid and atranorin were done checking their melting points and comparing their IR spectra with those given in the literature (Huneck and Yoshimura, 1996; Culberson, 1979). We determined that 100 ml of methanol, acetone, chloroform, and diethyl ether extracts contain 342, 99, 32, and 19 mg salazinic acid and 20, 45, 25, and 25 mg atranorin, respectively.

Determination of the MIC values of salazinic acid

The MIC value determination of salazinic acid against the bacteria, yeasts, and fungi were carried out the same way as described above for the determination of the MIC values of the extracts. First a stock solution of salazinic acid (12.0 mg/ml) in acetone was prepared. Then 2.5 ml of this stock solution were taken and diluted serially two-fold with acetone 4 times. From the stock solution and the diluted solutions, five sets of sixty filter paper discs containing salazinic acid in varying amounts were prepared. The salazinic acid contents of these discs varied from 500 μg/disc to 31.25 μg/disc.

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