Antimicrobial Activity of Extracts of the Lichen *Xanthoparmelia pokornyi* and its Gyrophoric and Stenosporic Acid Constituents

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The antimicrobial activity of the diethyl ether, acetone, chloroform, petroleum ether, and ethanol extracts of the lichen *Xanthoparmelia pokornyi* and its gyrophoric acid and stenosporic acid constituents has been screened against some foodborne bacteria and fungi. Both the extracts and the acids showed antimicrobial activity against *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Proteus vulgaris, Staphylococcus aureus, Streptococcus faecalis, Yersinia enterocolitica, Candida albicans* and *Candida glabrata*. The extracts were inactive against the tested filamentous fungi. The MIC values of the extracts and the acids for the bacteria have also been determined.

Key words: *Xanthoparmelia pokornyi*, Gyrophoric and Stenosporic Acid, Antimicrobial Activity

Introduction

Lichens perform a symbiotic relationship between algae and fungi and produce a great number of low molecular weight secondary compounds (Fahselt, 1994; Dayan and Romagni, 2001). The most comprehensive review for lichen compounds contains over 800 compounds of known structure (Huneck and Yoshimura, 1996). Lichen compounds are the metabolites of the fungal component of the lichens (Elix, 1996; Culberson and Culberson, 2001) and it has been proven that some of these compounds have biological and pharmaceutical activities including antibacterial, antifungal, antiviral and cytotoxic effects (Huneck, 1999, 2001; Perry *et al*., 1999; Manojlovic *et al*., 2002). Huneck (1999, 2001) presented lists of the lichen compounds and the lichens that show antibacterial and antifungal activities. Although the biological activities of lichen compounds have received considerable interest recently, the biological activities of a great number of lichen compounds are yet to be studied.

In this work, we investigated the antimicrobial activity of extracts of the lichen *Xanthoparmelia pokornyi* and its gyrophoric and stenosporic acid constituents. To the best of our knowledge, no information about the antimicrobial activities of an extract of *X. pokornyi*, gyrophoric acid and stenosporic acid is found in the literature, but only few studies about the biological activity of gyrophoric acid are reported. Gyrophoric acid was found to show antiproliferative and cytotoxic activity and inhibited the growth of the human keratinocyte cell line HaCaT with an IC_{50} value of 1.7 μM (Kumar and Müller, 1999a). It also significantly inhibited the light-dependent synthesis of ATP and uncoupled electron transfer on the reducing side of photosystem II in freshly lysed, illuminated spinach chloroplasts (Rojas *et al*., 2000). On the other hand this acid was inactive in the inhibition of leukotriene B_4 biosynthesis at concentrations up to 60 μM (Kumar and Müller, 1999b).

Experimental

Microorganisms

Aeromonas hydrophila and *Yersinia enterocolitica* (from Ankara University, Faculty of Veterinary, Ankara, Turkey), *Bacillus cereus* (NRRL B-3711), *Bacillus subtilis* (NRRL B-744), *Proteus vulgaris* (NRRL B-123), *Streptococcus faecalis* (NRRL B-14617), and *Geotrichum candidum* (NRRL Y-552) (from Northern Regional Research Laboratory of the USDA, Peoria, Illinois, USA), *Klebsiella pneumoniae*, *Cladosporium* sp., and *Salmonella typhimurium* (available in our department), *Listeria monocytogenes* (from Ankara

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University, Faculty of Agriculture, Ankara, Turkey), *Pseudomonas syringae* pv. *tomato* (TPPB 4212), *Alternaria tenuissima, Alternaria alternata, Alternaria citri* and *Penicillum notatum* (from Trakya University, Department of Biology, Edirne, Turkey), *Fusarium moniliforme*, *Fusarium oxysporum, Fusarium culmorum*, and *Cochliobolus sativus* (from the Agriculture Research Center, Eskişehir, Turkey), *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus,* and *Aspergillus parasiticus* (from Osmangazi University, Faculty of Medicine, Eskişehir, Turkey), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Aspergillus niger* (ATCC 9807) and *Fusarium solani* (ATCC 12820) (from American Type Culture Collection, USA) were used as microorganisms. Bacteria and yeasts were kept on nutrient agar and yeast extract agar plates at 4 °C, respectively. Fungal test cultures were subcultured on potato dextrose agar (PDA) for 5–7 d at 25 °C.

Lichen material

Xanthoparmelia pokornyi (Körb.) O. Blanco, A. Crespo, Elix, D. Hawksw. and Lumbsch was collected in Bozdağ, Eskişehir Province in Turkey at 1200 m on May 30, 2004. A voucher was stored at the Herbarium of Anadolu University in the Department of Biology (ANES).

Extraction

After adding air-dried and ground 10 g *X. pokornyi* samples to 100 ml of solvents of ethanol, acetone, diethyl ether, petroleum ether and chloroform, the mixtures were sonicated for 1 h, then left at room temperature overnight and filtered. The amounts of the extract residues in the filtrates were determined after removal of the solvents of 10 ml of the filtrates with a rotary evaporator.

Isolation and characterization of gyrophoric acid and stenosporic acid

First 100 ml acetone extract of 10 g of *X. pokornyi* was prepared as described above. After concentrating the extract to about 2 ml and spotting on preparative TLC plates coated with silica gel 60 F_{254} (20 × 20 cm, Merck), the plates were developed in solvent system B which consists of *n*-hexane, diethyl ether and formic acid (130:80:20, v:v:v) and is a solvent system used in the TLC of lichen substances (Orange *et al*., 2001; Huneck and Yoshimura, 1996). The spots belonging to gyrophoric acid (R_f 0.42) and stenosporic acid (R_f 0.72) on the developed plates were located using the R_f values of these compounds given in the literature (Huneck and Yoshimura, 1996; Orange *et al.*, 2001). The amounts of recovered gyrophoric acid and stenosporic acid were 18.0 mg and 126.3 mg, respectively. Besides the R_f values of these substances, their melting points and IR spectra were used for their further characterizations (Huneck and Yoshimura, 1996).

Determination of MIC values of the extracts

We employed the Kirby and Bauer disk diffusion method (National Committee for Clinical Laboratory Standards, 1993) to determine MIC values of the extracts of the lichen *X. pokornyi* against test bacteria and fungi. The loading of filter paper disks with the extract residues was done the same way as described in the literature (Yılmaz *et al.,* 2004; Tay *et al.,* 2004). From each extract, ten sets of sixty sterilized disks with varying amount of extract residue were prepared. The *X. pokornyi* extract residue contents of the disks varied from 1.76 mg/disk to 3.44 μg/disk for the ethanol extract, from 1.08 mg/disk to 2.11 μg/disk for the acetone extract, from 1.10 mg/disk to 2.15 μg/disk for the diethyl ether extract, from 0.04 mg/disk to 0.078 μg/disk for the petroleum ether extract, and 0.63 mg/disk to 1.23 μg/disk for the chloroform 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 disks 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 disks were used as negative control disks. 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.

Determination of MIC values of gyrophoric acid and stenosporic acid

The MIC value determinations of gyrophoric acid and stenosporic acid against the bacteria and

yeasts were carried out the same way as described above for the determination of the MIC values of the extracts. First stock solutions of gyrophoric acid (15.0 mg/ml) and stenosporic acid (12.5 mg/ml) in acetone were prepared. Then 2.0 ml of gyrophoric acid solution or 2.0 ml of stenosporic acid solution were taken from the stock solution and diluted two-fold with acetone nine times. Using these solutions, thirty filter paper disks containing gyrophoric acid or stenosporic acid in varying amounts were prepared. The substrate contents of these disks varied from 500 μg/disk to 1.95 μg/disk for gyrophoric acid and from 416 μg/disk to 1.63 μg/disk for stenosporic acid.

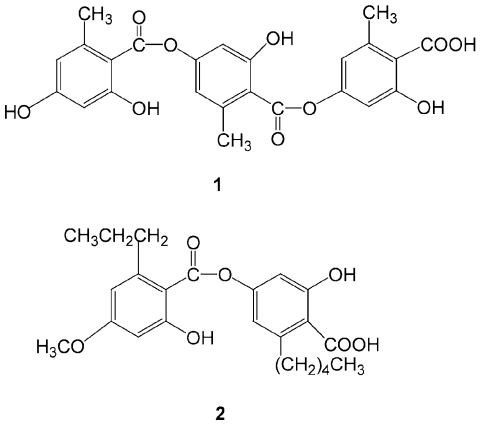

Fig. 1. Chemical structures of gyrophoric acid (**1**) and stenosporic acid (**2**).

Results and Discussion

In this study we screened the antimicrobial activity of the acetone, chloroform, ethanol, diethyl ether and petroleum ether extracts of the lichen *Xanthoparmelia pokornyi* and its gyrophoric and stenosporic acid constituents against thirteen Gram-positive and Gram-negative foodborne bacteria, two yeasts and fourteen filamentous fungi and determined their MIC values using the Kirby and Bauer disk diffusion method (Table I).

The screening results showed that the extracts of *X. pokornyi* have antibacterial activity against 8 out of 13 bacteria and antiyeast activity against 2 out of yeasts (Table I). No antifungal activity of the extracts against the filamentous fungi was detected. All extracts showed activity with different MIC values against the same microorganisms which are *A. hydrophila, B. cereus, B. subtilis, L. monocytogenes, P. vulgaris, S. aureus, S. faecalis, Y. enterocolitica, C. albicans* and *C. glabrata.* The extracts were inactive against *E. coli, K. pneumoniae, P. aeruginosa, P. syringae,* and *S. typhimurium* (Table I).

Table I. MIC values of the extracts of *Xanthoparmelia pokornyi* for bacteria and yeasts.

Microorganism	MIC[a] (against 10^7 cells [μg])				
	Ethanol extract[b]	Chloroform extract[b]	Petroleum ether extract[b]	Diethyl ether extract[b]	Acetone extract[b]
Aeromonas hydrophila	27.5	9.8	10.0	17.2	16.9
Bacillus cereus	27.5	19.7	5.0	34.4	33.8
Bacillus subtilis	27.5	19.7	5.0	17.2	16.9
Escherichia coli	–	–	–	–	–
Klebsiella pneumoniae	–	–	–	–	–
Listeria monocytogenes	55.0	39.4	10.0	17.2	8.4
Proteus vulgaris	55.0	39.4	2.5	17.2	16.9
Pseudomonas aeruginosa	–	–	–	–	–
Pseudomonas syringae	–	–	–	–	–
Salmonella typhimurium	–	–	–	–	–
Staphylococcus aureus	27.5	19.7	10.0	34.4	33.8
Streptococcus faecalis	55.0	19.7	10.0	17.2	16.9
Yersinia enterocolitica	55.0	19.7	10.0	34.4	33.8
Candida albicans	6.9	4.9	5.0	2.2	2.1
Candida glabrata	6.9	4.9	5.0	2.2	2.1

[a] All values are the results of duplicate experiments.
[b] The concentrations of the stock solutions were 5.28 mg/ml for the ethanol extract, 1.89 mg/ml for the chloroform extract, 1.20 mg/ml for the petroleum ether extract, 3.31 mg/ml for the diethyl ether extract, 3.24 mg/ml for the acetone extract.

Table II. MIC values of gyrophoric acid and stenosporic acid.

Microorganism	Gyrophoric acid		Stenosporic acid	
	[μg/66.7 μl][a]	[mM]	[μg/66.7 μl][a]	[mM]
Aeromonas hydrophila	125	4.00	208	7.50
Bacillus cereus	31.3	1.00	52.1	1.88
Bacillus subtilis	31.3	1.00	52.1	1.88
Escherichia coli	–		–	
Klebsiella pneumoniae	–		–	
Listeria monocytogenes	125	4.00	104	3.75
Proteus vulgaris	62.5	2.00	104	3.75
Pseudomonas aeruginosa	–		–	
Pseudomonas syringae	–		–	
Salmonella typhimurium	–		–	
Staphylococcus aureus	250	8.00	208	7.50
Streptococcus faecalis	125	4.00	52.1	1.88
Yersinia enterocolitica	500	16.0	416	15.0
Candida albicans	31.3	1.00	52.1	1.88
Candida glabrata	31.3	1.00	52.1	1.88

[a] MIC values are the results of duplicate experiments.

Although the obtained MIC values for all extracts were lower than 60 μg for each microorganism, we have had much lower MIC values for the same microorganisms in our previous studies with the extracts of the lichens *Ramalina farinacea* and *Cladonia foliacea* (Tay *et al*., 2004; Yılmaz *et al*., 2004).

To determine antimicrobial active major substances in the lichen *X. pokornyi*, we isolated and characterized stenosporic acid and gyrophoric acid constituents from the acetone extract of the lichen. The constituents of *X. pokornyi* given by Esslinger (1977) are stenosporic acid, gyrophoric acid and traces of divaricatic acid and unknown TE-1 or TE-3. Gyrophoric acid is a tridepside whereas stenosporic acid is a didepside (Fig. 1). Both acids were active or inactive against the same microorganisms as the extracts (Table II). The antibacterial activities of both acids were comparable to each other although their MIC values are reasonable higher than the MIC values of the extracts.

As a conclusion, this study is the first antimicrobial study on the extracts of *X. pokornyi* and its gyrophoric acid and stenosporic acid constituents.

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