Antimicrobial activity of extracts of the lichens *Cladonia furcata*, *Parmelia caperata*, *Parmelia pertusa*, *Hypogymnia physodes* and *Umbilicaria polyphylla*

B. RANKOVIĆ*, M. MIŠIĆ* and S. SUKDOLAK†
*Departments for *Biology and †Chemistry, Faculty of Science, University of Kragujevac, 34000 Kragujevac, Radoja Domanovicć 12, Serbia*

Accepted: 20 September 2007

ABSTRACT

The antimicrobial activity of acetone, methanol and aqueous extracts of the lichens *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogymnia physodes* and *Umbilicaria polyphylla* is assessed. The extracts are tested on six species of bacteria and 10 species of fungi using the disk-diffusion method, and broth tube dilution is used to determine minimal inhibitory concentration (MIC). The tested bacteria were more sensitive than the tested fungi. Aqueous extracts of the investigated lichens showed no antimicrobial activity against any of the test organisms, whereas the acetone and methanol extracts showed antimicrobial activity. In general, methanol extracts had stronger activity than did acetone extracts. The strongest activity was recorded for the methanol extract of *Parmelia pertusa*, which had the lowest measured MIC value (0.78 mg/mL). The least active species was *Parmelia caperata* (highest MIC value: 50 mg/mL). *Bacillus mycoides* was the most sensitive of the tested bacterial species, while *Candida albicans* was the most sensitive fungal species.

KEY WORDS: Antibacterial agents.
Antifungal agents.
Lichens.
Microbial sensitivity tests.

Introduction

As symbiotic organisms comprising a fungal and an algal component, lichens synthesise many metabolites known as lichen substances. These include aliphatic, cycloaliphatic, aromatic and terpenic compounds.[1] The metabolites produced by lichens can be classified into two groups, primary and secondary.[2] Primary metabolites are proteins, lipids, carbohydrates and other organic compounds. Some of these compounds are produced by the fungal component, and some by the algal component. Secondary metabolites are produced only by the fungal component. They are biosynthesised in hyphal cells, but later are deposited on the surface as crystals or amorphous layers.[3]

Lichens are found in extreme conditions, grow slowly and live for a long time. The specific conditions of their existence and slow growth account for the production of numerous components that protect them from various physical and biological influences.[4–7] Lichens and their metabolites have antiviral, antibiotic, antitumour, antiallergic, plant growth-inhibitory and enzyme-inhibitory activities.[8,9] Lichens also produce many secondary metabolites that inhibit the growth of microorganisms,[10–12] or protect them from ultraviolet radiation.[13] Different species have been used in traditional medicine to treat stomach ailments, diabetes, coughs, pulmonary tuberculosis and other diseases.[14–17]

It is known that microorganisms have developed resistance to many antibiotics. This creates enormous problems in the treatment of infectious disease, and investigators therefore seek new antimicrobial substances from different sources such as higher plants and lichens.[18–20] As secondary metabolites of lichens contain many active components, the purpose of the present study is to investigate their antimicrobial activity.

Correspondence to: Branislav Ranković
Department of Biology, Faculty of Science, University of Kragujevac, 34000 Kragujevac, Radoja Domanovića 12, Serbia
Email address: rankovic@kg.ac.yu

Here, the antimicrobial activity of extracts of the lichens *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogymnia physodes* and *Umbilicaria polyphylla* are tested against bacteria and fungi, including agents of human and animal diseases, causes of food spoilage and producers of mycotoxins.

Materials and methods

Lichens

Samples of the lichens *Cladonia furcata* (Huds.) Schrad., *Parmelia caperata* (L.) Ach., *Parmelia pertusa* (Hoffm.) Mass., *Hypogymnia physodes* (L.) Nyl. and *Umbilicaria polyphylla* (L.) Baumg. were collected on Mount Kopaonik during the summer of 2006. The demonstration samples are preserved in facilities of the Department of Biology and Ecology of Kragujevac University's Faculty of Science. Determination of the investigated lichens was accomplished using standard methods.[21–24]

Table 1. Antimicrobial activities of different extracts of *Cladonia furcata*, *Parmelia caperata*, *Parmelia pertusa*, *Hypogymnya physodes* and *Umbilicaria polyphylla* against the organisms tested using disk diffusion.

		B. m	B. s	E. cl	E. col	K. p	S. a	A. fl	A. fu	B. c	C. al	F. o	M. m	P. v	P. p	P. ver	T. h
	A	14±1[a]	12±0.58	14±1.53	-	12±1.53	15±2.52	12±2.52	12±1.53	10±1.53	10±2	11±1.53	-	13±1.53	12±1.53	10±1.53	12±0.58
C. furcata	B	15±0	12±1.53	15±0.58	-	12±1.53	15±2.52	13±1.53	15±1.53	10±0.58	10±1.53	12±0.58	-	15±1.53	12±0.58	12±2	12±1
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	-	-	-	-		-	10±2.08	9±1.53	18±1.53	18±1	12±1.53	-	8±0.58	7±1.53	18±0.58	12±1.73
P. caperata	B	19±1.53	15±2.52	-	17±1.53	14±2.52	16±0.58	16±0.58	15±1.53	33±1.53	23±1.53	32±1.53	-	27±1.73	24±1.53	27±1.15	24±0.58
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	27±0.58[b]	26±1.53	24±0.58	14±0.58	20±0.58	22±0	12±1.53	14±1.53	22±1.53	10±1.73	21±1.53	15±0.58	24±1.53	-	-	15±1.53
P. pertusa	B	19±1.53[b]	21±1.15	24±0.58	14±0.58	19±1.73	21±1.15[b]	15±1.53	17±1.15	23±1.53	17±2.08	21±1.15	15±0.58	25±2.08	18±0.58	-	18±1.53b
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	18±2.31	20±1.53	14±1.53	17±0.58	19±1	20±2.52[b]	14±0.58	15±1.53	20±0.58	15±1.73	19±1.15	-	12±0.58	14±2.08	10±0.58	10±1.53
H. physodes	B	17±2.31	20±1.53	15±1.53	18±1.53	14±1.53	18±0	16±1.15	14±1.15	20±0.58	15±0.58	16±0.58	-	13±0.58	13±1.53	10±0.58	10±1.53
	C	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
	A	-	-	-	-	-	-	14±0.58	10±2.52	16±1.53	12±1.15	12±1.15	15±1.53	-	-	12±0.58	-
U. polyphylla	B	15±1.53	15±2.52	17±1.53	-	16±2.31	-	20±1.53	30±1.53	22±2.08	10±1.15	22±1.53	25±0.58	-	20±2.08	22±2.08	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antibiotics	S	27±1.15	26±0.58	25±0.58	15±0.58	40±3.05	20±0.58	-	-	-	-	-	-	-	-	-	-
	K	-	-	-	-	-	-	27±2.08	34±2.08	39±1.15	40±3.21	35±2.08	17±1	40±0.58	38±1.15	36±1.53	18±0

A – acetone extract; B – methanol extract; C – aqueous extract

[a]Diameter of disk diffusion (mm) ± standard deviations. Diameter of inhibition zone (mm) including disk diameter of 7 mm.

Values are the mean of three replicate

[b]Statistical significance $P>0.05$; for all others the statistical significance was $P<0.05$.

Antibiotics: K – ketoconazole, S – streptomycin

B. m=*Bacillus mycoides*; B. s=*Bacillus subtilis*; E. cl=*Enterobacter cloaceae*; E. col=*E. coli*; K. p=*Klebsiella pneumoniae*; S. a=*Staphylococcus aureus*; A. fl=*Aspergillus flavus*; A. fu=*Aspergillus fumigatus*; B. c=*Botrytis cinerea*; C. al=*Candida albicans*; F. o=*Fusarium oxysporum*; M. m=*Mucor mucedo*; P. v=*Paecilomyces variotii*; P. p=*Penicillium purpurescens*; P. ver=*Penicillium verrucosum*; T. h=*Trichoderma harsianum*

Table 2. Minimum inhibitory concentration of *Cladonia furcata*, *Parmelia caperata*, *Parmelia pertusa*, *Hypogymnya physodes* and *Umbilicaria polyphylla* extracts against the test organisms.

		B. m	B. s	E. cl	E. col	K. p	S. a	A. fl	A. fu	B. c	C. al	F. o	M. m	P. v	P. p	P. ver	T. h
	A	0.78±0[a]	0.78±0	0.78±0	1.56±0	0.78±0	0.78±0	25±0	12.5±0	25±0	6.25±0	25±0	25±0	12.5±0	25±0	25±0	25±0
C. furcata	B	3.12±0	3.12±0	3.12±0	6.25±0	6.25±0	3.12±0	25±0	12.5±0	12.5±0	6.25±0	25±0	25±0	12.5±0	25±0	25±0	25±0
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	25±0	25±0	25±0	25±0	25±0	25±0	50±0	25±0	25±0	0.78±0	25±0	0.78±0	25±0	50±0	50±0	50±0
P. caperata	B	6.25±0	6.25±0	6.25±0	12.5±0	6.25±0	12.5±0	6.25±0	3.12±0	6.25±0	1.56±0	6.25±0	3.12±0	3.12±0	6.25±0	6.25±0	6.25±0
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	0.78±0	1.56±0	1.56±0	6.25±0	0.78±0	1.56±0	12.5±0	6.25±0	1.56±0	0.78±0	1.56±0	1.56±0	12.5±0	12.5±0	12.5±0	6.25±0
P. pertusa	B	0.78±0	0.78±0	0.78±0	6.25±0	0.78±0	1.56±0	6.25±0	3.12±0	1.56±0	0.78±0	1.56±0	1.56±0	6.25±0	6.25±0	6.25±0	6.25±0
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	3.12±0	6.25±0	1.56±0	6.25±0	3.12±0	3.12±0	25±0	12.5±0	3.12±0	3.12±0	12.5±0	12.5±0	6.25±0	12.5±0	12.5±0	25±0
H. physodes	B	3.12±0	3.12±0	3.12±0	6.25±0	1.56±0	3.12±0	12.5±0	6.25±0	3.12±0	3.12±0	6.25±0	6.25±0	3.12±0	6.25±0	6.25±0	6.25±0
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	1.56±0	1.56±0	3.12±0	1.56±0	1.56±0	6.25±0	12.5±0	12.5±0	6.25±0	1.56±0	12.5±0	12.5±0	6.25±0	12.5±0	12.5±0	12.5±0
U. polyphylla	B	0.78±0	0.78±0	0.78±0	1.56±0	0.78±0	1.56±0	1.56±0	1.56±0	1.56±0	1.56±0	1.56±0	1.56±0	1.56±0	1.56±0	1.56±0	1.56±0
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antibiotics	S	7.81±0	7.81±0	1.95±0	31.25±0	1.95±0	31.25±0	-	-	-	-	-	-	-	-	-	-
	K	-	-	-	-	-	-	3.9±0	3.9±0	1.95±0	1.95±0	3.9±0	31.25±0	1.95±0	3.9±0	3.9±0	7.81±0

A – acetone extract; B – methanol extract; C – aqueous extract

[a]Minimum inhibitory concentration (MIC) ± standard deviations; values given as mg/mL for lichen extracts and as µg/mL for antibiotics.

Statistical significance (*P*) not calculated.

Antibiotics: K – ketoconazole, S – streptomycin

B. m=*Bacillus mycoides*; B. s=*Bacillus subtilis*; E. cl=*Enterobacter cloaceae*; E. col=*E. coli*; K. p=*Klebsiella pneumoniae*; S. a=*Staphylococcus aureus*; A. fl=*Aspergillus flavus*; A. fu=*Aspergillus fumigatus*; B. c=*Botrytis cinerea*; C. al=*Candida albicans*; F. o=*Fusarium oxysporum*; M. m=*Mucor mucedo*; P. v=*Paecilomyces variotii*; P. p=*Penicillium purpurescens*; P. ver=*Penicillium verrucosum*; T. h=*Trichoderma harsianum*

Microorganisms and media

The bacteria used in this study were *Bacillus mycoides*, *B. subtilis* and *Staphylococcus aureus*, and *Enterobacter cloaceae, Escherichia coli* and *Klebsiella pneumoniae*. All were obtained from the Institute for Protection of Health in Kragujevac and the Faculty of Agriculture in Belgrade. Their identification was confirmed in the microbiological laboratory of Kragujevac University's Department of Biology. The fungi used as test organisms were *Aspergillus flavus* (ATCC 9170), *A. fumigatus* (DBFS 310), *Botrytis cinerea* (DBFS 133), *Candida albicans* (IPH 1316), *Fusarium oxysporum* (DBFS 292), *Mucor mucedo* (ATCC 52568), *Paecilomyces variotii* (ATCC 22319), *Penicillium purpurescens* (DBFS 418), *P. verrucosum* (DBFS 262) and *Trichoderma harsianum* (DBFS 379). These were obtained from the mycological collection maintained by the mycological laboratory within the Department of Biology of Kragujevac University's Faculty of Science. Bacterial cultures were maintained on Müller-Hinton agar substrates (Torlak, Belgrade). Fungal cultures were maintained on potato dextrose agar and Sabouraud dextrose (SD) agar (Torlak, Belgrade). All cultures were stored at 4°C and subcultured every 15 days.

Preparation of lichen extracts

Finely pulverised thalli of the investigated lichens (50 g) were extracted using acetone, methanol and water in a Soxchlet extractor. The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at –18°C until they were used in the tests. The extracts were dissolved in dimethyl sulphoxide (DMSO) for the disk-diffusion test. Minimal inhibitory concentration (MIC) was determined by preparing a series of dilutions in Müller-Hinton broth (for bacteria) or in SD broth (for fungi) in the range 0.05–50 mg/mL. The final concentration of DMSO did not exceed 2%.

Antimicrobial assays

The sensitivity of microorganisms to acetone, methanol and aqueous extracts of the investigated species of lichens was tested by measuring the zone of inhibition of a given concentration of extract by the disk-diffusion method and by determining the MIC.

Inoculi were obtained from bacterial cultures incubated for 24 h at 37°C on Müller-Hinton agar and diluted according to the 0.5 McFarland standard to approximately 10^8 colony-forming units (cfu)/mL.

Suspensions of fungal spores were prepared from fresh, mature (three to seven days old) cultures that grew at 30°C on a PDA substrate. Spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and then diluted to approximately 10^6 cfu/mL according to the procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS, M38-P, 1998).

A standard disk-diffusion method (NCCLS, M2-A5, 1993) was used to study antimicrobial activity. Müller-Hinton agar (for bacteria) or SD agar (for fungi) was seeded with the appropriate inoculum. Paper disks (7 mm diameter) were laid on the inoculated substrate after being soaked with 15 µL of lichen extract (50 mg/mL). Antimicrobial activity was determined by measuring the diameter of the zone of inhibition around the disk. Streptomycin (for bacteria) and ketoconazole (for fungi) were used as controls. A DMSO solution was used as a negative control for the influence of the solvents. All experiments were performed in triplicate.

The results obtained were processed statistically by standard deviation and to test for significance (*P* value).

The MIC was determined by the broth tube dilution method. A series of dilutions with concentrations ranging from 50 to 0.15 mg/mL was used in the experiment for each extract against all microorganism tested. The initial concentration of 50 mg/mL was obtained by measuring a quantity of extract and dissolving it in DMSO. Two-fold dilutions of extracts were prepared in Müller-Hinton for bacterial cultures and in SD broth for fungal cultures.

The MIC was determined by establishing visible growth of the microorganisms. The last dilution to show no visible growth was defined as the MIC for the tested microorganism at the given lichen extract concentration. Streptomycin and ketoconazole were used as positive controls. All experiments were performed in triplicate.

Results and discussion

The results of testing the antimicrobial activity of extracts of the investigated species of lichens are presented separately for the disk-diffusion (Table 1) and the broth tube dilution (Table 2) methods.

The antimicrobial activity of extracts of the lichens *Cladonia furcata*, *Parmelia caperata*, *P. pertusa* and *Umbilicaria polyphylla* is presented here for the first time.

Disk-diffusion method

On the basis of the results presented in Table 1, it can be seen that the lichen extracts showed strong antibacterial activity. Methanol and acetone extracts of *C. furcata, P. pertusa*, and *Hypogymnia physodes* showed significant antibacterial activity, whereas acetone extracts of *P. caperata* and *Umbilicaria polyphylla* were inactive.

With zones of inhibition comparable to those obtained with the streptomycin standard, the strongest activity against all bacteria tested was obtained with the acetone and methanol extracts of *P. pertusa*. The smallest zones of inhibition were seen with acetone and methanol extracts of *C. furcata*. The measured zones of inhibition were 12–15 mm in diameter.

Aqueous extracts of all investigated lichens failed to inhibit the tested bacteria. *Escherichia coli* showed the greatest resistance to the investigated lichen extracts. Similar antibacterial activity of related lichen species had been reported previously.[25–27]

All investigated lichens demonstrated antifungal activity against the tested fungal species. The inhibitory activity of acetone and methanol lichen extracts against the fungi tested was defined by inhibition zones in the range 7–33 mm. Aqueous extracts showed no inhibition. It is probable that active components with antimicrobial properties are insoluble or only very poorly soluble in water.[28]

With zones of inhibition comparable to those produced by the ketoconazole standard, the strongest inhibitory activity was obtained with the methanol extract of *P. caperata* against the fungi *Botrytis cinerea, Fusarium oxysporum* and *Paecilomyces variotii*. The other species showed lower but

approximately equal activity. *Mucor mucedo* showed the greatest resistance to the investigated extracts, being resistant to all but the acetone and methanol extracts of *Parmelia pertusa*. Among the tested fungi, the greatest sensitivity was shown by *B. cinerea*.

The values obtained for the action of the aqueous, acetone and methanol extracts were compared to those obtained with antibiotic agents. Statistical analysis showed that the difference was significant ($P<0.5$) for all values except those for the methanol extract of *P. pertusa* against *Staphylococcus aureus* and *Trichoderma harsianum*, as well as for the acetone extracts of *H. physodes* against *S. aureus* and of *P. pertusa* against *Bacillus mycoides* and *B. subtilis* ($P>0.05$).

Minimal inhibitory concentration

Extracts of all investigated lichens showed antibacterial activity. The MICs were in the range 0.78–25 mg/mL for different extracts. The acetone extract of *C. furcata* had the strongest inhibitory activity. At a concentration of 0.78 mg/mL, it inhibited five of the tested bacteria. The weakest activity was shown by extracts of *P. caperata*, especially its acetone extract, which inhibited the tested bacteria at a concentration of 25 mg/mL.

Extracts of *P. pertusa, H. physodes* and *U. polyphylla* showed approximately the same inhibitory activity towards the tested bacteria, but the methanol extracts exerted stronger inhibitory action than did the acetone extracts. *E. coli* showed the greatest resistance to the investigated extracts. The most sensitive bacteria were *B. mycoides, B. subtilis* and *Klebsiella pneumoniae*. In general, Gram-negative bacteria were more resistant than Gram-positive bacteria. This difference in sensitivity can be ascribed to morphological differences between the microorganisms, and above all to differences in permeability of the cell wall.[29] Aqueous extracts manifested no activity in relation to the tested microorganisms.

Extracts of the lichens showed selective antifungal activity, with MIC values in the range 0.78–50 mg/mL. Aqueous extracts did not show any inhibitory activity. The strongest antifungal activity was found in the methanol extract of *U. polyphylla*, which inhibited all of the tested fungi at a concentration of 1.56 mg/mL. Weaker activity was demonstrated by extracts of *C. furcata* and *P. caperata*, in which MIC values fluctuated over the range 6.25–50 mg/mL.

Extracts of *P. pertusa* and *H. physodes* showed approximately equal antifungal activity, with methanol extracts exerting a stronger inhibitory action than did the acetone extracts. Among the tested fungi, the greatest sensitivity was shown by *Candida albicans*.

As a negative control, DMSO, used in the same concentrations as for the lichen extracts, showed no inhibiting effect on the tested organisms. Used as positive controls, streptomycin ketoconazole inhibited growth of all the tested bacteria and fungi, respectively.

In investigations of the antimicrobial activity of lichen extracts conducted by other authors, there are both similarities and differences with the results presented here. Halama and van Haluwin[30] obtained similar results when studying the antifungal activity of lichen extracts (including extracts of *H. physodes*) against phytopathogenic fungi. Gulluce *et al*.[26] found that a methanol extract of *P. saxatilis* had stronger antibacterial activity than antifungal activity. Candan *et al*.[31] established antimicrobial activity for different extracts of the lichen *Xanthoparmelia pokornyi* against bacteria and yeasts, but not against filamentous fungi. In contrast, Shahi *et al*.[32] found that extracts of *P. cirrhatum* exhibited strong antifungal activity against filamentous fungi.

The results presented here indicate that the methanol extract of *U. polyphylla* manifested the strongest antimicrobial activity. Earlier investigations of the antimicrobial activity of methanol extracts of the related *U. nylanderiana* also showed strong antimicrobial activity.[26]

These similarities and differences in the antimicrobial activity of extracts of different lichen species probably are a consequence of the presence of different components with antimicrobial activity. The results presented here indicate that the investigated extracts manifest strong but varying antimicrobial activity, which suggests that extracted components from various lichens may prove useful in treating many diseases caused by microorganisms. □

This work was financed partially by the Ministry of Science, Technology and Development of the Republic of Serbia and is the result of studies in Project 143041B.

References

1 Huneck S, Yoshimura Y. *Identification of lichen substances*. Berlin: Springer-Verlag, 1996.
2 Elix JA. Biochemistry and secondary metabolites. In: Nash TH ed. *Lichen biology*. Cambridge: University Press, 1996: 154–80.
3 Öztürk S, Güvenç Ş, Arikan N, Yýlmaz Ö. Effect of usnic acid on mitotic index in root tips of *Allium cepa L. Lagascalia* 1999; **21**: 47–52.
4 Lawrey JD. Lichen secondary compounds: evidence for a correspondence between antiherbivore and antimicrobial function. *Bryologist* 1989; **92**: 326–8.
5 Herbert RA. A perspective on the biotechological potential of extremophiles. *Trends Biotechnol* 1992; **10**: 395–402.
6 Lauterwein M, Oethinger M, Belsner K, Peters T, Marre R. *In vitro* activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid and (–)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrob Agents Chemother* 1995; **39**: 2541–3.
7 Tay T, Özdemir Türk A, Yilmaz M, Türk H, Kivanç M. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstictic acid, and protocetraric acid constituents. *Z Naturforsch C* 2004; **59**: 384–8.
8 Lawrey JD. Biological role of lichen substances, 1986; *Bryologist* **89**: 111–22.
9 Huneck S. The significance of lichens and their metabolites. *Naturwissenschaften* 1999; **86**: 559–70.
10 Gollapudi SR, Telikepalli H, Jampani HB *et al*. Alectosarmentin, a new antimicrobial dibenzofuranoid lactol from the lichen *Alectoria sarmentosa. J Nat Prod* 1994; **57**: 934–8.
11 Ingólfsdottir K, Chung GAC, Skúlason VG, Gissurarson SR, Vilhelmsdóttir M. *In vitro* antimycobacterial activity of lichen metabolites. *Eur J Pharm Sci* 1998; **6**: 141–4.
12 Cocchietto M, Skert N, Nimis PL, Sava G. A review usnic acid, an interesting natural compound. *Naturwissenschaften* 2002; **89**: 137–46.
13 Fernandez E, Quilhot W, Gonzales I, Hidalgo ME, Molina X, Meneses I. Lichen metabolites as filters. *Cosmet Toiletries* 1996; **111**: 69–74.
14 Vartia KO. Antibiotics in lichens. In: Ahmadjian V, Hale ME eds. *The lichens*. New York: Academic Press, 1973: 547–61.

15 Richardson DHS. Medicinal and other economic aspects of lichens. In: Galun M ed. *Handbook of lichenology* (Vol. 1). Boca Raton: CRC Press, 1988: 93–108.
16 Baytop T. Therapy with medicinal plants in Turkey (past and present). Istanbul University, Istanbul, 1999.
17 Ingólfsdottir K, Hjalmarsdóttir MA, Sigurdsson A, Gudjónsdóttir G-A, Brynjólfsdóttir A, Steingrimsson O. *In vitro* susceptibility of *Helicobacter pylori* to protolichesterinic acid from the lichen *Cetraria islandica*. *Antimicrob Agents Chemother* 1997; **41**: 215–7.
18 Mitscher L, Drake S, Gollapudi SR, Okwute SK. A modern look at folkloric use of anti-infective agents. *J Nat Prod* 1987; **50**: 1025–40.
19 Crittenden PD, Porter N. Lichen-forming fungi: potential sources of novel metabolites. *Trends Biotechnol* 1991; **9**: 409–14.
20 Karaman I, Sanin F, Güllüce M, Ogütcü H, Sengul M, Adigüzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxicedrus*. *J Ethnopharmacol* 2003; **37**: 1–5.
21 Purvis OW, Coppins BJ, Hawksworth DL, James PW, Moore DM. The lichen flora of Great Britian and Ireland. London: Natural History Museum (in association with the British Lichen Society), 1992.
22 Wirth V. Die Flechten. Baden-Wurttembergs, Teil 1 & 2. Stuttgart: Eugen Ulmer, 1995.
23 Dobson F. *Lichens. An illustrated guide*. England: Richmond Publishing, 2000.
24 Poelt J, Vezda A. Bestimmungsschlüssel Europäischer Flechten, Erganzunsheft II. Bibliotheca Lichenologica. Vaduz: Cramer, 1981.
25 Madamombe IT, Afolayan AJ. Evaluation of antimicrobial activity of extracts from South African *Usnea barbata*. *Pharmaceut Biotechnol* 2003; **41**: 199–202.
26 Gulluce M, Aslan A, Sokmen M *et al*. Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis, Platismatia glauca, Ramalina pollinaria, Ramalina polymorpha* and *Umbilicaria nylanderiana*. *Phytomedicine* 2006; **13**: 515–21.
27 Ranković B, Mišić M. Antifungal activity of extract of the lichens *Alectoria sarmentosa* and *Cladonia rangiferina*. *Mikol Fitopatol* 2007; **41**: 276–81.
28 Kinoshita K, Matsubara H, Koyama K *et al*. Topics in the chemistry of lichen compounds. *J Hattori Bot Lab* 1994; **76**: 227–33.
29 Nostro MP, Germanó V, D'Angelo A, Marino, Cannatelli MA. Extraction methods and bioautography for evaluation of medical plant antimicrobial activity. *Lett Appl Microbiol* 2000; **30**: 379–84.
30 Halama P, van Haluwin C. Antifungal activity of lichen extracts and lichenic acids. *Bio Control* 2004; **49**: 95–107.
31 Candan M, Yilmaz M, Tay T, Kivanc M. Antimicrobial activity of extracts of the lichen *Xanthoparmelia pokornyi* and its gyrophoric and stenosporic acid constituents. *Z Naturforsch (C)* 2006; **61**: 319–23.
32 Shahi SK, Patra M, Dikshit A, Upreti DK *Parmelia cirrhatum*: a potential source of broad-spectrum natural antifungal. *Phytother Res* 2003; **17**: 399–400.