Antimicrobial activity of some lichens and their components

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Abstract: - Antibacterial and antifungal activity of the acetone, methanol and aqueous extracts of the lichens Lecanora frustulosa and Parmeliopsis hyperopta and their divaricatic acid and zeorin constituents has been screened in vitro against the following species of microorganisms: Bacillus mycoides, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Aspergillus flavus, Aspergillus fumigatus, Botrytis cinerea, Candida albicans, Fusarium oxysporum, Mucor mucedo, Paecilomyces variotii, Penicillium purpureascens, Penicillium verrucosum, Trichoderma harsianum. The antimicrobial activity was estimated by the disc-diffusion method and determination of the minimal inhibitory concentration (MIC) by the Broth tube Dilution method. The bacteria were more sensitive related to the tested fungi. Acetone and methanol extracts of the investigated lichens showed relatively strong antimicrobial activity, whereas aqueous extracts showed no antimicrobial activity against any of the test organisms. Divaricatic acid and zeorin also showed strong activity against bacteria and fungi. There was no antimicrobial activity against Escherichia coli species. Generally, the tested lichens extracts and lichens compounds demonstrated a strong antimicrobial effect against the tested microorganisms. That suggest a possibility of their use in a treatment of various diseases caused by these and similar microorganisms.

Key-words: - Antimicrobial activity; Lichens extracts; Lichens components

Introduction
Lichens are symbiotic associations between fungi and algae [1]. They synthesise various secondary metabolites of a “lichen components” [2]. Up to now about 350 components are known from lichens and approximately 200 have been characterized [3]. They are extracellular products of relatively low molecular weight crystallized on the hyphal cell walls. Also they are usually insoluble in water and can be extracted into organic solvents [4]. They make even more than 30% of the dry mass of talus [5]. Various biological activities of some lichens and their components are known, such as: antiviral, anti-tumor, anti-inflammatory, analgetic, antipirethic, antiproliferative, antiprotosoal [6,7,8,9]. Besides, many sorts are used for human nutrition, animal nutrition, for getting colours, perfumes, alcohol and in the medicine industry. [10,11,12,13]. Several lichens species have been used in folk medicine for treatment of stomach deseases, diabetes, whooping cough, pulmonary tuberculosis, cancer treatment, skin deseases [14, 9,12]. It has been proven that the lichen extracts and lichen components have a distinguished antimicrobial activity [15,16]. Because of that, the purpose of this work is to investigate in vitro the antimicrobial activity of the acetone, methanol and aqueous extract of the chosen lichens and their components in relation to a
Finely ground thalli of the investigated lichens (50 g) were extracted using acetone, methanol and water in a Soxhlet 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. Lichen substances were isolated from the obtained extracts by the method of preparative chromatography (TLC – silica gel G in the system of the solvents toluene: dioxane: vinegar acid = 90 : 25 : 4). The mixtures were identified by the chemical and physical-chemical analysis (tt. Rf (TLC), IR, H1- NMR i MS – mass spectrum). The isolated lichen components: divaricatic acid from the lichen Lecanora frustulosa and zeorin from the lichen Parmeliopsis hyperopta. The extracts and acids were dissolved in dimethyl sulphoxide (DMSO). Minimal inhibitory concentration (MIC) was determined by preparing a series of dilutions in Müller-Hinton broth (for bacteria) or in SD broth (for fungy). The final concentration for the DMSO didn’t extend 2% in the experiment.

Antimicrobial assays
The sensitivity of microorganisms to acetone, methanol, aqueous extracts of the investigated species of lichens and their component was tested by measuring the zone of inhibition of a given concentration of extract by the disk diffusion method and by determining the minimal inhibitory concentration (MIC). Bacterial inoculi were obtained from bacterial cultures incubated for 24 h at 37°C on Müller-Hinton agar substrate and brought up by dilution according to the 0.5 McFarland standard to approximately 10^8 CFU/ml. Suspensions of fungal spores were prepared from fresh mature (3- to 7-day-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 further diluted to approximately 10^6 CFU/ml according to the procedure recommended by the [20].

A standard disk-diffusion method [21] was used to study antimicrobial activity. Müller-Hinton agar (for bacteria) or in SD agar (for fungy) 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 minimal inhibitory concentration (MIC) was determined by the broth tube dilution method. A series of dilutions with concentrations ranging from 50 to 0.195 mg/mL for extracts and 25 to 0.097 mg/mL for components was used in the experiment against every microorganism tested. The starting solutions of extracts and component were obtained by measuring off a certain quantity of extract and dissolving it in DMSO. Two-fold dilutions of extracts and components were prepared in Müller-Hinton broth for bacterial cultures and SD broth for fungal cultures in test tubes. The minimal inhibitory concentration was determined by establishing visible growth of the microorganisms. The boundary dilution without any visible growth was defined as the minimal inhibitory concentration (MIC) for the tested microorganism at the given concentration. As a positive control of growth inhibition, streptomycin was used in the case of bacteria, ketoconazole in the case of fungi. All experiments were performed in triplicate.

Results

The antimicrobial activity of the tested lichen extracts and lichen acids against the tested microorganisms was shown in the tables, for lichen extracts (Table 1) and for lichen acid (Table 2).

Disc-diffusional method

The acetone and methanol extracts of the tested lichens showed relatively strong antimicrobial activity, whereas aqueous extracts were inactive. The extracts of the lichen Lecanora frustulosa inhibited five out of six tested bacteria. The zones of inhibition were large. The largest zones of inhibition were recorded with the methanol extract, especially relative to the species Bacillus mucoides (24 mm). The antifungal activity of these extracts was selective, the acetone extract inhibited two and methanol extract seven of the tested fungal species. The zones of inhibition were within the range 13-17 mm.

The acetone and methanol extracts of the lichen Parmeliopsis hyperopta showed strong antibacterial activity on all of the tested bacteria except Escherichia coli, which was resistant. The zones of inhibition relative to the bacteria were within the range 11 – 18 mm for the acetone and 11 – 21 mm for the methanol. Extracts of this lichen showed a strong antifungal activity on all of the tested fungi. The measured zones of inhibition were also large. The strongest activity was found in the methanol extract against Paecilomyces variotii (26 mm).

Minimal inhibitory concentration (MIC)

The MIC for the different extracts related to the tested bacteria and fungi were within the range 0.78-12.5 mg/mL. The biggest antimicrobial activity was in the extracts of the lichen Parmeliopsis hyperopta. The acetone and methanol extract of this lichen had approximately equal antibacterial activity, but the methanol extract exerted stronger antifungal activity than did acetone extract. The measured MIC values were within the range 0.78-1.56 mg/mL related to the bacteria and 1.56-12.5 mg/mL related to the fungi.

The extracts of the lichen Lecanora frustulosa showed selectively antimicrobial activity, although it should be stressed that the methanol extract had shown a stronger inhibitory influence than the acetone. The MIC for the acetone and methanol extracts of this lichen were within the range 0.78-3.12 mg/mL related to the bacteria and 3.12-12.5 mg/mL related to the fungi.

Divaricatic acid from lichen Lecanora frustulosa and zeorin from lichen Parmeliopsis hyperopta demonstrated very strong antimicrobial activity, with the bacteria demonstrating bigger sensitivity than the fungi. Zeorin showed stronger antibacterial activity than did divaricatic acid. At a concentration of 0.39 mg/mL, zeorin inhibited four out of six tested bacteria. Divaricatic acid demonstrated antibacterial activity in the concentrations 0.78 mg/mL and 1.56 mg/mL. The MIC for both components relative to the fungi were within the range 3.12-12.5 mg/mL.

Discussion

In this paper, for the first time, has been presented the antimicrobial activi
Table 1. Antimicrobial activities of acetone, methanol and aqueous extracts of *Lecanora frustulosa* and *Parmeliopsis hyperopta* against tested microorganisms based on disc-diffusion and broth tube dilution methods

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Lecanora frustulosa</th>
<th>Parmeliopsis hyperopta</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td><strong>DD&lt;sup&gt;b&lt;/sup&gt;-MIC&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>DD-MIC</td>
<td>DD-MIC</td>
<td>DD-MIC</td>
</tr>
<tr>
<td>Bacillus mycoides</td>
<td>22-1.56</td>
<td>24-0.78</td>
<td>- -</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>13-3.12</td>
<td>15-1.56</td>
<td>- -</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>12-1.56</td>
<td>17-0.78</td>
<td>- -</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>19-1.56</td>
<td>23-0.78</td>
<td>- -</td>
</tr>
<tr>
<td>Staphilococcus aureus</td>
<td>18-1.56</td>
<td>21-0.78</td>
<td>- -</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>- -</td>
<td>15-12.5</td>
<td>- -</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>14-12.5</td>
<td>17-3.12</td>
<td>- -</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>- -</td>
<td>14-6.25</td>
<td>- -</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>- -</td>
<td>15-12.5</td>
<td>- -</td>
</tr>
<tr>
<td>Mucor mucedo</td>
<td>- -</td>
<td>13-12.5</td>
<td>- -</td>
</tr>
<tr>
<td>Paecilomyces variotii</td>
<td>14-12.5</td>
<td>16-6.25</td>
<td>- -</td>
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<tr>
<td>Penicillium purpurencens</td>
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<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Penicillium verrucosum</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Trichoderma harsianum</td>
<td>- -</td>
<td>16-3.12</td>
<td>- -</td>
</tr>
</tbody>
</table>

<sup>a</sup> A – acetone extract; B – methanol extract; C – aqueous extract.

<sup>b</sup> Diameter of inhibition zone (mm) including disc diameter of 7 mm.

<sup>c</sup> Minimum inhibitory concentration (MIC); values given as mg/ml for lichen extract and as µg/ml for antibiotics.

Values are the mean of three replicate

Antibiotics: K – ketoconazole, S – streptomycin
Table 2. Minimum inhibitory concentration (MIC) of divaricatic acid and zeorin against the test organisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>B. m</th>
<th>B. s</th>
<th>E.cl</th>
<th>E. coli</th>
<th>K. p</th>
<th>S. a</th>
<th>A.fl</th>
<th>A. fu</th>
<th>B. c</th>
<th>C. al</th>
<th>Fu. o</th>
<th>M. m</th>
<th>Pe. ve</th>
<th>P. pur</th>
<th>Pen. v</th>
<th>T.h</th>
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<tbody>
<tr>
<td><strong>Lichen compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divaricatic acid</td>
<td>1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56</td>
<td>0.78</td>
<td>-</td>
<td>0.78</td>
<td>1.56</td>
<td>12.5</td>
<td>12.5</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>12.5</td>
<td>12.5</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>Zeorin</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td>-</td>
<td>0.39</td>
<td>0.78</td>
<td>6.25</td>
<td>6.25</td>
<td>3.12</td>
<td>3.12</td>
<td>3.12</td>
<td>6.25</td>
<td>3.12</td>
<td>6.25</td>
<td>6.25</td>
<td>3.12</td>
</tr>
<tr>
<td>S</td>
<td>7.81</td>
<td>7.81</td>
<td>1.95</td>
<td>31.25</td>
<td>1.95</td>
<td>31.25</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.9</td>
<td>3.9</td>
<td>1.95</td>
<td>1.95</td>
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<td>31.25</td>
<td>1.95</td>
<td>3.9</td>
<td>3.9</td>
<td>7.8</td>
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</table>

<sup>a</sup>Minimum inhibitory concentration (MIC); values given as mg/ml for lichen extract and as µg/ml for antibiotics

**Antibiotics:** K – ketaconazole, S – streptomycin
extracts of the lichens Lecanora frustulosa and Parmeliopsis hyperopta and their divaricatic acid and zeorin constituents. The tested lichen extracts and lichen acid show a relatively strong antimicrobial activity. The intensity of the antimicrobial effect depended on the sort of the extract, its concentration and the tested microorganism. Similar differences were also noticed by other investigators [16]. The aqueous extracts of the tested lichens didn’t show any antimicrobial activity. That’s probably because the active components produced by lichens are either insoluble or poorly soluble in water. [22]. The antibacterial effect is stronger relative to the antifungal. These results could be expected considering the fact that numerous tests proved that bacteria are more sensitive to the antibiotics compared with fungi [23]. The reason of different sensitivity between the fungi and bacteria can be found in different transparency of the cell wall [24]. The cell wall of the gram-positive bacteria consists of peptidoglycans (mureins) and teichoic acids, the cell wall of the gram-negative cells consists of lipo polysaccharides, and lipopoliproteins [25,26,27] whereas the cell wall of fungi consists of polysaccharides such as hitchin and glucan [28,29].

Previous researches showed significant bioactive characteristics of similar lichens. [15] found out that the methanol extract of the lichen P. saxatilis had a strong antimicrobial influence. Similar results were reported by [30] for different extracts extracted from the lichen Parmelia sulcata and its salazinic acid constituent [31] find an antimicrobial activity for the extracts of the lichens Parmelia caperata and Parmelia pertusa.

The obtained results showed that the tested lichen extracts and lichen acids showed a significant antimicrobial activity relative to the tested bacteria and fungi, which could be of significance in therapy human, animal and plant diseases. Further studies should be done to search new compounds from lichens that exhibit strong antimicrobial activity.

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References


