

Antibacterial and Antifungal Activities of Gelatinose and non-Gelatinose Lichen Species

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Abstract

Background: Despite the wide diversity of the basic growth forms (crustose, squamulose, foliose, and fruticose), all lichens have a similar internal morphology. The bulk of the lichen's body is formed from filaments of the fungal partner, and the relative density of these filaments defines the layers within the lichen.

Objectives: To continue and complete the previous study, this study was designed to assess the antibacterial and antifungal activity of different extractions of lichens.

Materials and Methods: Acetone, methanol and aqueous extractions of the lichens, including *Acarospora strigata*, *Collema crispum*, *Placidium squamulosum*, *Physcia biziana*, *Lecanora prophetae-eliae* and *Ramalina farinacea*, were evaluated in vitro against seven bacterial strains (*Escherichia coli* ATCC1652, *Salmonella typhi* ATCC1679, *Proteus mirabilis* ATCC2601, *Staphylococcus aureus* ATCC1885, *Enterococcus faecalis* ATCC2321, *Staphylococcus epidermidis* ATCC2405, and *Bacillus cereus* ATCC13061), and two fungi (*Verticillium dahlia*, *Fusarium moniliforme*). The antibacterial activity was estimated via disc diffusion method and the minimal inhibitory concentration (MIC) was determined via broth tube dilution method.

Results: Among the six tested lichens, methanol extracts of *P. squamulosum*, *P. biziana*, and *L. prophetae-eliae* showed relatively high antibacterial activities and also the acetone extraction of *L. prophetae-eliae* showed antibacterial activity against *S. epidermidis* and *B. cereus*. The bacteria were more sensitive than the fungi. The methanol extract of *P. squamulosum* showed the highest antibacterial activity; besides, the least amount of MIC value was 250 mg/mL.

Conclusions: It seems that the tested lichens could be effective as antibiotics; especially, in terms of drug resistance, they can be proper substitutes; but, further studies are suggested.

Keywords: Antibacterial Activity, Antifungal Activity, Lichens

1. Background

In the food industry, the biological activity of plant and lichen extracts has been investigated for the possible use of essential oils for the control of pathogenic bacteria. This property is entirely based on the presentation of various biological components including steroidal glycosides, polyhydroxylated sterols, naphthoquinone pigments, and antibacterial peptides (1, 2).

Lichens are a result of a specific symbiotic relation between algal and fungal partners. They synthesize various secondary metabolites called "lichen components", mostly from the fungal metabolism (3). To date, approximately 350 lichen substances have been known and about 200 have been characterized (4), which are extracellular products from modest molecular weights, crystallized on the hyphal cell walls (5). They make even more than 30% of the dry mass of the thallus (6). Some lichens and their phenolic components have various biological properties such as antiviral, antitumor, anti-inflamma-

tory, analgesic, antipyretic, and antiprotozoal (7-10). Several lichen species have distinguished antibacterial and antifungal properties and they have been used in traditional medicine (7, 11-13). Based on topographical, ecological and floristic criteria, Ilam province in Iran with 160 lichen species is divided into two main zones, each of which is divided into four parts as follows: 1- the Nubosindian zone, including: a) desert, b) stabilized dunes, c) river beds, and d) shrublands, hills, and badlands; 2- the Kurdo-Zagrosian zone, including: a) degraded *Quercus* forest, mostly cultivated by indigenous people, b) rocky, calcareous and deep slopes bearing the *Quercus* forest vegetation, c) shrubby parts above the *Quercus* tree-line, and d) high mountains with low-lying vegetation (14-20).

2. Objectives

The aim of this study was the assessment of in vitro antibacterial and antifungal activities of different lichen

extracts against specific microorganisms that cause diseases in humans, animals and plants. No reports are available on antibacterial properties of the chosen lichens from Iran (Iranian lichens) and most of the samples collected from Ilam province (part 2 as Kurdo-Zagrosian zone) were investigated.

3. Materials and Methods

3.1. Lichen Samples

This study was based on non-gelatinose *Ramalina farinacea* samples previously collected from Mazandaran province, Iran (20). Other specimens collected from Ilam province during summer 2011 were identified as *Lecanora prophetae-eliae*, *Acarospora strigata*, *Physconia distorta*, *Placidium squamulosum* (non-gelatinose lichens), and *Collema crispum* (gelatinose lichen) in the previous study (21).

3.2. Microorganisms

Seven bacterial strains including *Escherichia coli* ATCC1652, *Staphylococcus aureus* ATCC1885, *Enterococcus faecalis* ATCC2321, *Salmonella typhi* ATCC1679, *Staphylococcus epidermidis* ATCC2405, *Bacillus cereus* ATCC13061, and *Proteus mirabilis* ATCC2601 were procured from the mycological collection maintained in the mycological laboratory inside the department of microbiology, Tehran university of medical sciences. The fungi used as test microorganisms (*Fusarium moniliforme* and *Verticillium dahlia*) were obtained from the Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran.

3.3. Preparation of Lichen Extracts

The air-dried powders of lichens (50 g) were extracted in the form of water, acetone, and methanol (250 mL) using Soxhlet extractor. The extracts were filtered by Whatman filter paper (No. 1) and then concentrated under reduced pressure in a rotary evaporator. The obtained residues were stored at -18°C until used in the tests. Then, they were dissolved in 5% (v/v) dimethyl sulfoxide.

3.4. Screening of Antimicrobial Activity

The suspensions of fungal spores were prepared from

fresh mature cultures (3 - 7-day-old) grown at 30°C on a potato dextrose agar (PDA) substrate. Spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and further diluted to approximately 106 CFU/mL (CFU: colony-forming units) according to the procedure recommended by the national committee for clinical laboratory standards (NCCLS) (M 38 - P, 1998). Bacterial inoculi were obtained from bacterial cultures incubated for 24 hours at 37°C on Muller-Hinton agar substrate and brought up by dilution according to the 0.5 McFarland standards to approximately 108 CFU/mL.

Muller-Hinton agar (for bacteria) or sabouraud dextrose agar (for fungi) was seeded with the appropriate inoculum. Being soaked with 2.5 - 37 mg/mL of lichen extract, paper discs (6 mm) were laid on the inoculated substrate. Antibacterial and antifungal activities were determined via standard disc diffusion method [Kosanic and Rankovic (22)], by assaying the diameter of the inhibition zone around the disc. Streptomycin in the case of bacteria and ketoconazole (10 µg/mL) in the case of fungi were used as positive controls. Sterile distilled dimethyl sulfoxide (DMSO) was used as negative control. Determination of the minimal inhibitory concentration (MIC) was done by broth tube dilution method (63 - 1000 mg/mL). All the experiments were performed in triplicate.

3.5. Statistical Analysis

All the statistical analyses were performed using SPSS version 13 statistical software package. The data were analyzed by one-way analysis of variance (ANOVA). P values < 0.01 were considered statistically significant. All the results were expressed as mean ± SD for three experiments in each.

4. Results

The methanol extract of *P. squamulosum* was effective in all tested Gram-positive and Gram-negative bacteria. The higher zone of inhibition was associated with *B. cereus* ATCC13061 in 1000 mg/mL concentration. The methanol extract of *C. crispum* inhibited four bacteria including *E. coli* ATCC1652, *S. aureus* ATCC1885, *B. cereus* ATCC13061, and *E. faecalis* ATCC2321 (Table 1).

Table 1. The Antibacterial Activity of Methanol Extracts^a

Bacteria	Lichens											
	<i>C. crispum</i>		<i>P. squamulosum</i>		<i>L. prophetae-eliae</i>		<i>A. strigata</i>		<i>P. biziana</i>		<i>R. farinacea</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>S. aureus</i> ATCC1885	1000	1000	500	1000	125	250	n	n	n	n	n	n
<i>S. epidermidis</i> ATCC2405	n ^b	n	250	500	125	250	n	n	n	n	n	n
<i>E. faecalis</i> ATCC2321	1000	1000	500	1000	500	500	n	n	n	n	n	n
<i>E. coli</i> ATCC1652	1000	1000	500	1000	250	150	n	n	n	n	n	n
<i>S. typhi</i> ATCC1679	n	n	250	500	n	n	n	n	n	n	n	n
<i>P. mirabilis</i> ATCC2601	n	n	250	500	n	n	n	n	n	n	n	n
<i>B. cereus</i> ATCC13061	1000	1000	1000	1000	250	250	n	n	n	n	n	n

^aAbbreviations: MBC, minimum bacterial concentration; MIC, minimum inhibitory concentration.

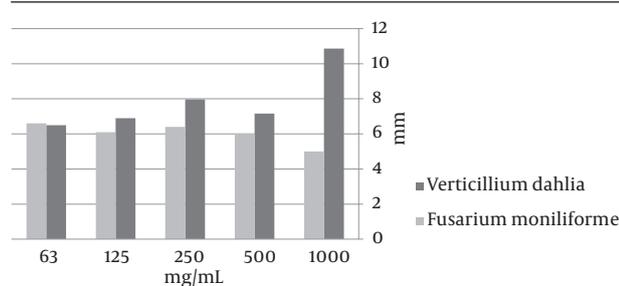
^bn, inactive.

Table 2. Effect of Acetone Extract of *Lecanora prophetae-eliae* on Tested Bacteria

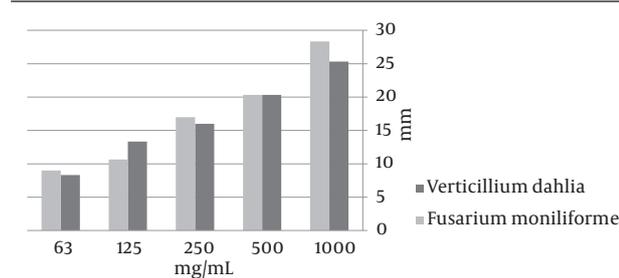
Bacteria	34/3 ^a	13/6	4/9	2/1	0/8
<i>B. cereus</i> ATCC13061	19.12 ± 0.09 ^b	15.07 ± 0.01	14 ± 0.04	12.73 ± 0.06	8.33 ± 0.01
<i>S. epidermidis</i> ATCC2405	21 ± 0.02	17.54 ± 0.03	12.33 ± 0.09	10.84 ± 0.02	11 ± 0.01
<i>E. coli</i> ATCC1652	-	-	-	-	-
<i>S. typhi</i> ATCC1679	-	-	-	-	-
<i>P. mirabilis</i> ATCC2601	-	-	-	-	-
<i>S. aureus</i> ATCC1885	-	-	-	-	-
<i>E. faecalis</i> ATCC2321	-	-	-	-	-

^aAbsorbed extract by discs in different concentrations (mg/mL).

^bAverage diameter of the inhibition zone of the acetone extract of gelatinose and non-gelatinose lichens.

Figure 1. Average Diameters of the Inhibited Zones

They were resulted from the effects of methanol extract of *P. squamulosum* in concentration of 63 - 1000 mg/mL on *V. dahlia* and *F. moniliforme*.

Figure 2. Average Diameters of the Inhibited Zones

They were resulted from the effects of the methanol extract of *C. crispum* on *V. dahlia* and *F. moniliforme*.

Methanol extracts of *A. strigata*, *P. distorta* and *R. farinacea* besides aqueous extracts of all the tested lichens were inactive against all the tested microorganisms. The acetone extract of *L. prophetae-eliae* showed antibacterial activity against *S. epidermidis* ATCC2405 and *B. cereus* ATCC13061 (Table 2).

The methanol extracts of *C. crispum* and *P. squamulosum* showed antifungal properties, but *C. crispum* was more effective than *P. squamulosum* and the effect of *P. squamulosum* against *F. moniliforme* was not significant (Figures 1 and 2).

5. Discussion

The results of this study showed that aqueous extrac-

tions had no antimicrobial activity and this was probably because the active components produced by lichens were a little diluted (20). Methanol extracts of *P. squamulosum* showed a high antibacterial activity against all seven tested bacteria. Acetone extract of the species did not show any antibacterial activity. Among the fungi, the most sensitive one against the methanol extract of *C. crispum* was *F. moniliforme* (with zones of inhibition ranging from 8 to 20 mm).

There is no report concerning the antibacterial properties of *P. squamulosum*, *L. prophetae-eliae*, and *C. crispum* in the world and in this study, the methanol extracts of these species showed high antibacterial activities and further study for the detection of secondary lichen products is needed.

Bacteria are more sensitive against antibiotics than fungi and it might be related to the different transparency of the cell wall (23). Gram-positive cell wall consists of peptidoglycan (murein) and teichoic acids and Gram-negative cells consist of lipopolysaccharides and lipopolyproteins (24, 25), whereas the hyphal cell wall of fungi consists of polysaccharides such as hitchin and glucan (23, 26). Similar results were reported by Shyam Kumar et al. for different extractions from the *C. auriforme* (27). Kosanic et al. (26) found an antibacterial activity for the extractions of the *C. cristatum*. In a study by Jeon et al. *A. strigata* showed inhibition of more than 80% in mycelial growth of a pathogen (28).

According to the results of this study, the methanol extracts of the tested lichens had significant antimicrobial effects on some microorganisms. Therefore, it can be beneficial in treatment of some diseases caused by tested microorganisms; especially, in terms of drug resistance they can be proper substitutes, but further studies are suggested.

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Footnotes

Authors' Contribution:The first author participated in design of the study and preparation of the manuscript,

also conducted experiments on antimicrobial activity of tested species. The second author adopted the manuscript to the Journal's instructions to authors and submitted the manuscript.

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