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Original Article

Antibacterial and Antifungal Activities of Different Lichens Extracts

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Introduction: Natural products have been the most successful source of potential drug leads. One of them is lichens, which have been used since antiquity as natural sources of drugs. The aim of this study was to investigate the antibacterial and antifungal activity of different extracts of the Iranian lichens in Ilam Province. **Methods:** The aqueous, acetone, and methanol extracts of 6 lichen species, including *Caloplaca variabilis*, *Fulgensia subbracteata*, *Lecanora muralis*, *Physcia adscendens*, *Psora decipiens,* and *Megaspora verrucosa*, were produced using Soxhlet extractor. Then, antibacterial and antifungal activities of them against 6 standard strains of bacteria, including *Enterococcus faecalis* ATCC 2321, *Escherichia coli* ATCC 1652, *Proteus mirabilis* ATCC 2601, *Salmonella typhi* ATCC 1679, *Staphylococcus aureus* ATCC 1885, and *Staphylococcus epidermidis* ATCC 2405 and two fungi, including *Fusarium moniliforme* and *Verticillium dahliae,* were evaluated using the disc-diffusion method and the minimal inhibitory concentration was determined by broth tube dilution. Streptomycin (10 µg/ml) and ketoconazole (10 µg/ml) were used as positive controls for bacteria and fungi, respectively. Sterile distilled dimethyl sulfoxide (DMSO) was used as negative control. **Results:** Methanol extract from *F. subbracteata* and *L. muralis* lichens showed relatively high antibacterial activities ($p<0.01$), whereas aqueous extracts showed no activity against the microorganisms and only acetone extract of *L. muralis* showed antibacterial activity against the *S. epidermidis* (MIC=500). Methanol extracts of *F. subbracteata* and *L. muralis* had higher antifungal activities than others. **Conclusion:** The methanol extracts of *L. muralis* and *F. subbracteata* showed the highest activity against the bacteria and fungi and it seems that they have some antibacterial and antifungal properties. *J Med Microbiol Infec Dis, 2014, 2 (2): 71-75.*

Keywords: Antibacterial, Antifungal, Extracts, Lichens.

INTRODUCTION

Due to increasing microbial resistance to commonly used antibiotics and reduced effectiveness of common and reliable standard drugs, achievement of natural bioactive compounds has been paid more attention [1]. One of the biological compounds could be lichens, which have been used as natural sources of drugs since antiquity [2]. Lichens are a mutualistic symbiont consisting of a fungus (the mycobiont) and an alga or/and cyanobacteria (the photobiont) [3]. They have been used in traditional medicines for centuries. Up to now, about 200 characterized components have been identified from 13.500 species of described lichen-forming fungi in the world [4, 5]. They have various exclusive biological activities, such as antimicrobial, antifungal (especially against pathogen fungi), antiviral, antiprotozoal anti-proliferative, anti-tumor, antioxidant, anti-inflammatory, analgesic, and antipyretic activities [1, 6-8]. Lichens are valuable resources for food, dyes, perfumes, and spices. Some of them are also used in folk medicine and treatment of various diseases, such as stomach diseases, diabetes, cough, pulmonary tuberculosis, wounds healing, and dermatological diseases [9].

Ilam Province, in the southwest of Iran, is home to about 160 lichen species [10-15]. There are a few reports on antibacterial properties of lichens from Ilam and other parts of Iran. Therefore, the aim of this study was to investigate the antibacterial and antifungal activities of aqueous, acetone and methanol extracts from the lichens collected from Ilam Province.

MATERIAL AND METHODS

Lichen materials. Fresh lichens were collected from Dare Arghavan area [Lat. 33º 39' 18" N. Long. 46º 26' 12" E., alt: 600 m] in Ilam Province, Iran during December 2011. The morphology of all specimens was studied using a stereomicroscope. The species were identified using standard spot tests solutions (K, C, KC, and Pd). Six species of lichens were identified, including *Caloplaca variabilis* (Pers.) Müll. Arg., *Fulgensia subbracteata* (Nyl.) Poelt, *Lecanora muralis* (Schreb.) M. Choisy., *Physcia adscendens* (Fr.) H. Olivier, *Psora decipiens* (Hedw.) Hoffm., and *Megaspora verrucosa* (Ach.) Hafellner & V. Wirth. Identification of the lichens was done using a standard key [16].

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Preparation of lichen extracts. The lichen samples were shade dried in a well-ventilated place at room temperature. Then, the materials were ground by an electric grinder. Chemical constituents were extracted from 50 g of ground lichens using one of solvents, including methanol, acetone, and distilled water using a Soxhlet [17]. The extracts were concentrated by evaporation in an oven at 35-40°C. The dry extracts were stored at -18°C for further experimental assays. The test extracts were dissolved in 2% dimethyl sulfoxide (DMSO).

Microorganisms. In this study, the following bacteria and fungi were used as test organisms: *Enterococcus faecalis* ATCC2321, *Escherichia coli* ATCC1652, *Proteus mirabilis* ATCC2601, *Salmonella typhi* ATCC1679, *Staphylococcus aureus* ATCC1885, *Staphylococcus epidermidis* ATCC2405, *Fusarium moniliforme* and *Verticillium dahlia.* All used bacteria were obtained from the stock culture of Microbiology Research Laboratory of Ilam University, Iran.

Antibacterial assays. A standard disc-diffusion method was used to assay the antibacterial activity. Müller-Hinton agar (in the case of bacteria) and potato dextrose agar (in the case of fungi) were seeded with the appropriate inoculum. Paper discs (6 mm diameter) were placed on the inoculated substrate after being soaked with 15 µl of lichen extract (50 mg/ml). A series of dilutions with concentrations ranging from 63 mg/ml to 1000 mg/ml was used for the extracts. Antibacterial activity was determined by measuring the diameter of the inhibition zone around the disc. Streptomycin (10 µg/ml) and ketoconazole (10 µg/ml) were used as positive controls for bacteria and fungi, respectively. Sterile distilled DMSO was used as negative control. Minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the extracts were determined according to the procedure described previously [17]. All assays were performed in triplicate.

Statistical analysis. 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, each in triplicate.

RESULTS

Aqueous extracts of all tested lichens showed no significant antibacterial activity. Also, methanol extract of four lichens, including: *C. variabilis, P. decipiens, P. adscendens*, and *M. verrucosa* did not show antibacterial properties (p>0.01). All acetone extracts except that of *L. muralis* showed no activity (figure 1).

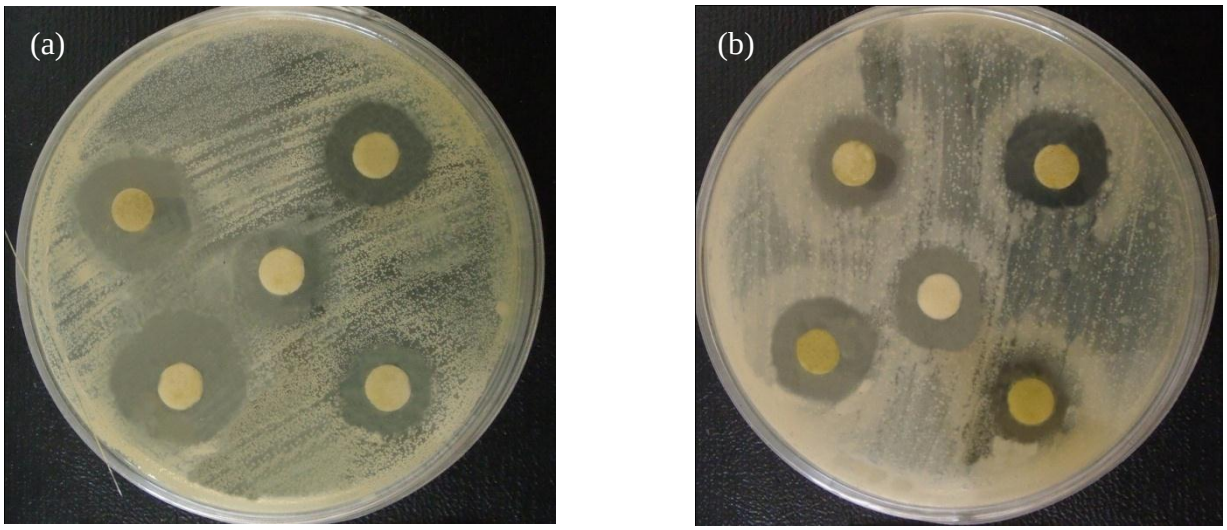


Fig. 1. Disc diffusion of acetone extracts of *L. muralis* against (a) *S. epidermidis* and (b) *E. coli*

The bacteria *S. typhi* ATCC1679 and *P. mirabilis* ATCC2601 were resistant to the methanol extract of *L. muralis and F. subbracteata,* but the four other bacteria showed more sensitivity against the extract compared to standard antibiotics tested, and *E. faecalis* was resistant to *F. subbracteata* extract, as shown in Table 1.

The methanol extract of *L. muralis* (1000 mg/ml dilution) showed the largest zone of inhibition (34 mm) against the *S. aureus* ATCC1885 and the least zone of inhibition (19 mm) was observed against *E. faecalis* ATCC2321. However, streptomycin as a positive control for bacteria showed the largest zone of inhibition (17 mm) against *E. faecalis* ATCC2321. Acetone extract of only *L. muralis* was effective against *S. epidermidis* ATCC2405 (Table 2).

Three species, including *F. subbracteata, C. variabilis,* and *L. muralis* showed high antifungal activity (Figures 2, 3, and 4 and Table 3).

The results of the two tested fungi showed that the methanol extract (1000 mg/ml) of *L. muralis* was effective only against *V. dahlia*, but *C. variabilis* and *F. subbracteata* were effective against both tested fungi. The methanol extract (1000 mg/ml) of *F. subbracteata* (MIC=125) showed the highest antifungal activity. The methanol extract of *C. variabilis* showed an antifungal activity but no antibacterial activity (Table 3).

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Table 1. Antibacterial activity (MIC and MBC mg/ml) of methanol extract of *F. subbracteata* and *L. muralis*

	F. subbracteata		*L. muralis*	
	MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
***S. aureus* ATCC1885**	250	250	125	250
***S. epidermidis* ATCC2405**	500	500	125	250
***E. faecalis* ATCC2321**	n	n	500	500
***E. coli* ATCC1652**	500	500	250	250
***S. typhi* ATCC1679**	n	n	n	n
***P. mirabilis* ATCC2601**	n	n	n	n

n: no effect

Table 2. Effect of acetone extract of *L. muralis* on *S. epidermidis*

		L. muralis				
		63 mg/ml	**125 mg/ml**	**250 mg/ml**	**500 mg/ml**	**1000 mg/ml**
***S. epidermidis* ATCC2405**	Absorbed extracted by discs	34.3	13.6	4.9	2.1	0.8
	Average diameter of inhibition zone (m.m)	19±0.01	16±0.03	12.33±0.01	10.67±0.07	9±0.05

Table 3. Average diameter of inhibition zone of methanol extract of lichens (1000 mg/ml) against fungi

	C. variabilis	*F. subbracteata*	*L. muralis*	*P. decipiens*
F. moniliforme	27.33±0.02	30.33±0.01	-*	-
V. dahlia	24.33±0.01	34.33±0.02	22.33±0.04	-

* Inactive

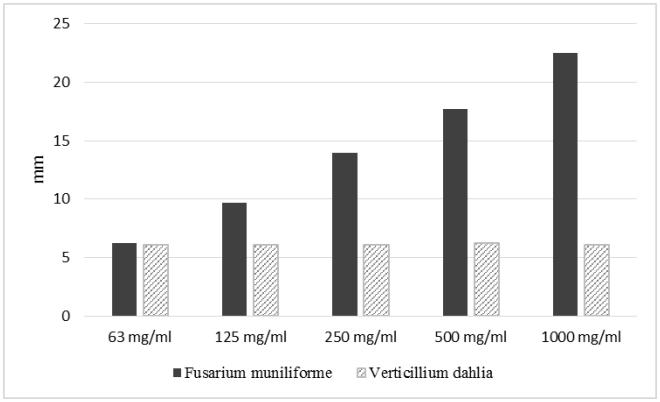


Fig. 2. Average diameter of inhibition zone of methanol extracts of *L. muralis* in concentrations of 63 to 1000 mg/ml against *V. dahlia* and *F. monilifore*

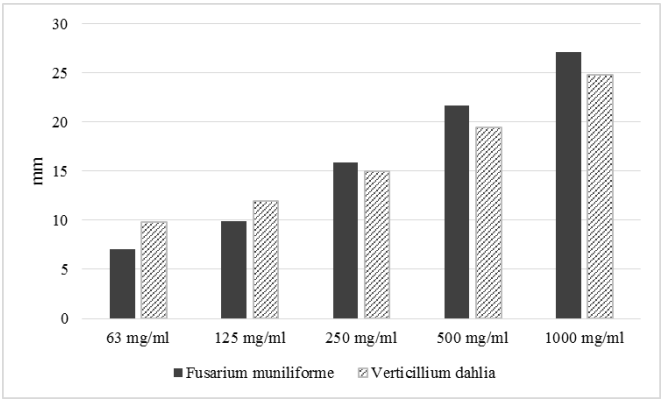


Fig. 3. Average diameter of inhibition zone of methanol extracts of *C. variabilis* in concentrations of 63 to 1000 mg/ml against *V. dahlia* and *F. moniliforme*

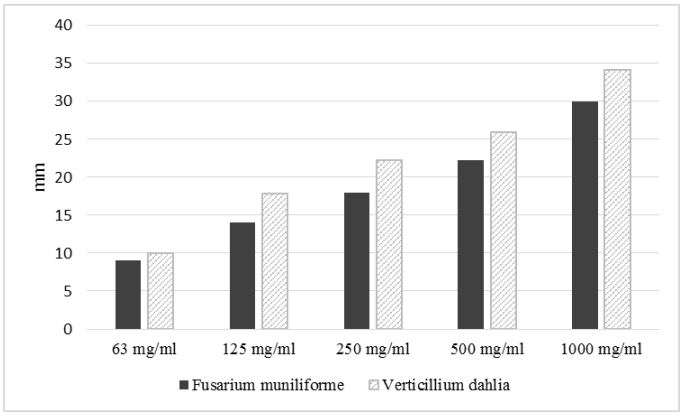


Fig. 4. Average diameter of inhibition zone of methanol extract of *F. subbracteata* in concentrations of 63- 1000 mg/ml against *V. dahlia* and *F. moniliforme*

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DISCUSSION

According to the results of this study and other similar researches [6-7, 9], the methanol extracts of lichens showed a higher antibacterial activity against gram-positive bacteria than gram-negative bacteria. Also, the antibacterial activity was higher than antifungal activity. It seems that differences in morphology, pore, permeability, and transparency of the cell wall of microorganisms may be the reason for differences in sensitivity between bacteria and fungi and even between gram-positive and gram-negative bacteria [18-20]. The cell wall of gram-positive bacteria consists of peptidoglycans (mureins) and teichoic acids. The cell wall of the gram-negative consists of lipopolysaccharides, and lipoproteins, whereas the cell wall of fungi consists of polysaccharides, such as hitchin and glucan [18-20].

The resistance of the majority of microorganisms against various antibiotics is growing and consequently causes difficulty in the treatment of infectious diseases [21]. The results of the current study showed that lichen extracts could be effective against bacteria and fungi, and the methanol extracts of some lichens have higher antibacterial activity than standard antibiotics [19, 20, 22]. *L. muralis* showed the highest antibacterial activity against *S. aureus* than the others, while only acetone extracts of *L. muralis* showed antibacterial activity.

The differences in the results of some studies may be due to differences in geographical areas [18]. Hence, the obtained MIC values for *L. muralis* in this study appear to be higher than those reported in the literature from other countries [18]. Also, in other studies, *F. subbracteata* has shown no inhibitory activity against bacteria [23]. However, in our study, the lichen species from Iran showed a strong antimicrobial activity against the tested microorganisms. In general, few reports are available on antimicrobial properties of Iranian lichens (*e.g., F. fulgense* [12] and *Parmotrema* sp. [23]). Therefore, further studies should be conducted to investigate other properties of Iranian lichens and their chemical compounds that are responsible for their antibacterial activity [20, 22].

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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