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Research article/Araştırma makalesi

Investigation of the antifungal activity of some epiphytic and terricolous lichen extracts

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Abstract

Antifungal activity of two terricolous (*Cetraria aculeata*, *Cladonia foliacea*) and four epiphytic (*Parmelia sulcata*, *Pseudevernia furfuracea* var. *furfuracea*, *Ramalina farinacea* and *Tornabea scutellifera*) lichens were investigated by using acetone, methanol and chloroform extracts against five fungal pathogens: *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Fusarium moniliforme*, *Fusarium solani* and *Alternaria brassicicola*. The microbroth dilution method was used for minimal inhibitory concentration (MIC) values of the lichen extracts. The MIC values of the lichen extracts were determined ranging from 156.25 to1250 µg/ml for tested fungi. Generally, all of the lichen extracts showed the strong antifungal activity. *F. moniliforme* and *A. brassicicola* were the most sensitive fungal species against the lichen extracts, whereas *A. fumigatus* was the most tolerant pathogenic fungus.

Key words: : antifungal activity, lichen extracts, pathogenic fungi

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Bazı epifitik ve terrikol liken ekstraktlarının antifungal aktivitesinin araştırılması

Özet

İki terrikol (*Cetraria aculeata*, *Cladonia foliacea*) ve dört epifitik (*Parmelia sulcata*, *Pseudevernia furfuracea* var. *furfuracea*, *Ramalina farinacea* ve *Tornabea scutellifera*) likenin aseton, metanol ve kloroform ekstraktlarının *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Fusarium moniliforme*, *Fusarium solani* ve *Alternaria brassicicola* fungal patojenlerine karşı antifungal aktivitesi incelenmiştir. Liken ekstraktlarının minimum inhibisyon konsantrasyonu (MİK) değerlerinin belirlenmesinde mikrobroth dilüsyon yöntemi kullanılmıştır. Liken ekstraktlarının MİK değerlerinin, test edilen funguslar için 156.25 ila 1250 µg/ml aralığında değiştiği belirlenmiştir. Genel olarak, liken ekstraktlarının tamamı güçlü antifungal aktivite göstermiştir. *F. moniliforme* ve *A. brassicicola* liken ekstraktlarına karşı en duyarlı fungal türler olarak belirlenirken, *A. fumigatus* en dirençli patojen fungus olarak bulunmuştur.

Anahtar kelimeler: antifungal aktivite, liken ekstraktları, patojenik funguslar

1. Introduction

Lichens are symbiotic organisms consisting of fungi, algae and/ or cyanobacteria. They produce various secondary metabolites known as lichen substances, are mostly low molecular weight and generally insoluble in water but can be extracted into organic solvents. Their secondary metabolites have a wide various biological activities such as antibacterial, antifungal, antiviral, antiprotozoal, antiherbivore, antimutagenic, antitumor, antioxidant, antiulcerogenic, antiinflammatory, antiproliferative, antipyretic, analgesic (Kosanić and Ranković, 2011; Mitrović et al., 2011; Xu et al., 2016).

Plant pathogenic fungi cause huge economic loss worldwide by destroying crops in the field and during storage in agriculture and the food industry (Goel et al., 2011; Song et al., 2017). Numerous environmental and human health problems are caused by synthetic chemicals used for control of these pathogenic fungi in agriculture (Halama and Van Haluwin, 2004). Natural products obtained from plants, bacteria, fungi, lichens etc. have been proposed as potential alternatives to synthetic fungicides for control of pre- and postharvest diseases. Environmentally safe, biodegradable

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and economical compounds obtained from natural origin can be ideal candidates for use as agrochemicals (Goel et al., 2011).

There are only a few reports on antifungal activities of lichens from Turkey (Aslan et al., 2006; Güllüce et al., 2006; Türk et al., 2006; Candan et al., 2007; Karabulut and Özturk, 2015; Yılmaz Cankılıç et al., 2017; Bilgin Sökmen et al., 2018). The aim of the present study is to investigate antifungal activity of the acetone, methanol and chloroform extracts of the lichens *Cetraria aculeata, Cladonia foliacea, Parmelia sulcata, Pseudevernia furfuracea* var. *furfuracea, Ramalina farinacea* and *Tornabea scutellifera* against five plant pathogenic fungi.

2. Materials and methods

2.1. Lichen samples

The lichen samples were collected from as follows; *Cetraria aculeata* (Schreb.) Fr., Bozdağ Eskişehir; *Cladonia foliacea* (Huds.) Willd., Bozdağ-Eskişehir; *Parmelia sulcata* Taylor, Bozdağ-Eskişehir; *Pseudevernia furfuracea* var. *furfuracea* (L.) Zopf., Mihalıççık-Eskişehir; *Ramalina farinacea* (L.) Ach., Bozdağ-Eskişehir and *Tornabea scutellifera* (With.) J.R. Laundon, Çevrepınar mountain-Kahramanmaraş (Figure 1). The samples were identified using standard keys (Smith, 2009; Wirth, 2013). The lichen specimens were deposited in the Department of Biology, Herbarium of Anadolu University (ANES), Eskişehir, Turkey.

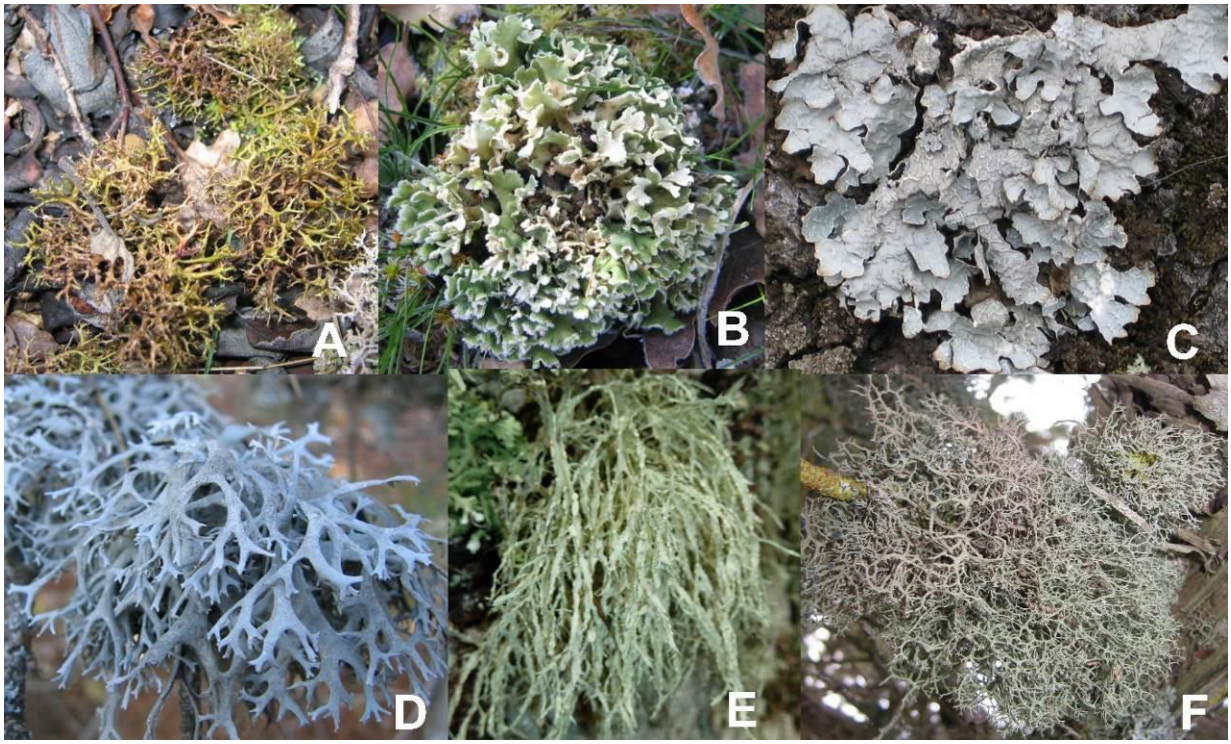

Figure 1. Lichen samples: A. *Cetraria aculeata,* B. *Cladonia foliacea,* C. *Parmelia sulcata,* D. *Pseudevernia furfuracea* var. *furfuracea,* E. *Ramalina farinacea,* F. *Tornabea scutellifera.*

2.2. Preparation of the lichen extracts

Air-dried and ground thalli of the lichen samples (10 g) were extracted in 100 ml of acetone, methanol and chloroform using ultrasonic bath for 30 min, then left at room temperature overnight and filtered. Then the extracts concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at 4 °C.

2.3. Microorganisms and media

Five fungal strains were used in this study: *Aspergillus fumigatus* NRRL 113, *Aspergillus parasiticus* NRRL 465, *Alt*

ernaria brassicicola, *Fusarium moniliforme* NRRL 2374 and *Fusarium solani*. The strains obtained from our laboratory. Fungal test cultures were grown on Potato Dextrose agar (PDA) at 25 °C for 5-7 days.

2.4. Antifungal activity

Minimal inhibitory concentration (MIC) of the lichen extracts was determined by microbroth dilution method using 96-well microtiter plate (Winn, 2006; CLSI, 2008). The extracts were dissolved in dimethyl sulphoxide (DMSO) and two-fold dilutions prepared from 10 mg/mL to 19.53 µg/mL concentrations in double-strength Potato Dextrose broth (PDB). Fungal cultures were grown on PDA at 25 °C for 5-7 days. The suspensions of fungal spores were prepared using sterile 0.1% Tween 80 and standardized to about 10^6 colony forming unit / ml (CFU/ml). Ketoconazole used as a positive control of the fungal growth inhibition. As a negative control DMSO solution was used for the influence of the solvents. The microplates were incubated at 25 °C for 5-7 days. Antifungal activity was determined with stereo microscope by investigation of mycelia growing. MIC defined as the lowest concentration that inhibited fungal growth. All experiments were performed in duplicate..

3. Results

The antifungal activities of the acetone, methanol and chloroform extracts of *C. aculeata*, *C. foliacea*, *P. sulcata*, *P. furfuracea* var. *furfuracea*, *R. farinacea* and *T. scutellifera* against the tested five filamentous fungi were investigated and results are shown in Table 1. The antifungal activity of the lichens extracts were determined by the microbroth dilution method. The MIC values of the lichen extracts were found ranged from 156.25 to1250 µg/ml for tested fungi.

Table 1. Minimal inhibitory concentration (MIC) of acetone, methanol and chloroform extracts of *C. aculeata, C. foliacea, P. sulcata, P. furfuracea var. furfuracea, R. farinacea* and *T. scutellifera* against selected fungi

Lichens	**Lichen extracts**	**Filamentous fungi**				
		A. fumigatus	*A. parasiticus*	*A. brassicicola*	*F. moniliforme*	*F. solani*
Cetraria aculeata	A[a]	625[b]	625	156.25	156.25	1250
	M	625	625	156.25	156.25	1250
	C	625	312.50	156.25	156.25	625
Cladonia foliacea	A	625	625	156.25	156.25	625
	M	1250	625	312.50	156.25	625
	C	1250	625	156.25	156.25	625
Parmelia sulcata	A	625	625	156.25	156.25	625
	M	625	312.50	156.25	156.25	312.50
	C	625	312.50	156.25	156.25	312.50
Pseudevernia furfuracea var. *furfuracea*	A	625	312.50	156.25	156.25	312.50
	M	625	312.50	156.25	156.25	156.25
	C	1250	625	156.25	156.25	156.25
Ramalina farinacea	A	312.50	625	156.25	156.25	625
	M	625	625	156.25	156.25	156.25
	C	625	625	156.25	156.25	625
Tornabea scutellifera	A	625	625	156.25	156.25	1250
	M	625	625	156.25	156.25	625
	C	625	625	312.50	156.25	625
Antifungal (standard)						
Ketaconazole		78.12	78.12	78.12	39.06	39.06

[a] A: acetone extract; M: methanol extract; C: chloroform extract

[b] Minimum inhibitory concentration (µg/ml)

The acetone extracts of *R. farinacea* showed the stronger antifungal activity against *A. fumigatus* with an MIC value of 312.50 µg/ml than the other tested lichen extracts. The methanol and chloroform extracts of the lichen *P. furfuracea* var. *furfuracea* and methanol extracts of *R. farinacea* exerted the stronger antifungal activity against *F. solani* than the other lichens extracts with an MIC value of 156.25 µg/ml.

All the lichen extracts showed same activity against to *F. moniliforme* and *A. brassicicola* with an MIC value of 156.25 µg/ml except methanole extract of *C. foliacea* and acetone extract of *T. scutellifera*. Generally, *A. fumigatus* was the most resistant fungi against the lichen extracts. *F. moniliforme* and *A. brassicicola* were the most sensitive fungi among the fungal species by lichen extracts.

Candan et al. (2007) investigated the antimicrobial activities of acetone, chloroform, diethyl ether, methanol, and petroleum ether extracts of the lichen *P. sulcata* and its salazinic acid constituent, and all of the extracts except of the petroleum ether extract showed antimicrobial activity against tested bacteria, yeasts and filamentous fungi including *Aspergillus niger, Aspergillus fumigatus,* and *Penicillium notatum*. In the present study, the methanol and chloroform extracts of *P. sulcata* showed significantly antifungal activity among the test fungi ranged from 156.25 to 625 µg/ml.

Türk et al. (2006) reported antifungal activity of some extracts of *P. furfuracea* var. *furfuracea* against tested filamentous fungi. All the extracts showed antifungal activity against *Alternaria alternata*, *Ascochyta rabiei*, *Aspergillus niger*, *Fusarium culmorum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium solani*, and *Penicillium notatum*. We determined the most strongest antifungal activity on *F. moniliforme*, *F. solani* and *A. brassicicola* for extracts of *P. furfuracea* var. *furfuracea*.

Ranković et al. (2007) reported antifungal activity on *A. fumigatus* with an MIC value of 25 mg/ml for acetone and methanol extracts of *P. sulcata*. Kosanić et al. (2013) find an antifungal activity for the acetone extract of the lichen *P. furfuracea* against same fungus with an MIC value of 12.5 mg/ml. In our study, we determined more activity than their results using acetone and methanol extracts of same lichen against *A. fumigatus* with an MIC value 625 µg/ml (Table 1).

In a similar study, Karabulut and Özturk (2015) was reported that ethyl alcohol extracts of *Evernia prunastri*, *P. sulcata* and *P. furfuracea* var. *furfuracea* showed a significant inhibition against mycelia and spor growth of *Aspergillus niger, Botrytis cinerea, Fusarium culmorum, Fusarium solani*, *Macrophomina phaseolina, Penicillium expansum* and *Rhizoctonia solani*.

4. Conclusions and discussion

The extracts of *C. aculeata, C. foliacea, P. sulcata*, *P. furfuracea* var. *furfuracea*, *R. farinacea* and *T. scutellifera* were exhibited significantly antifungal activity against tested pathogenic fungi. Future studies should be done to search bioactive compounds responsible for antifungal effects in the lichen extracts. Our results indicated these lichen extracts can be used as natural antifungals especially against some plant pathogenic fungi in future.

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References

Aslan, A., Güllüce, M., Sökmen, M., Adigüzel, A., Sahin, F., Özkan, H. (2006). Antioxidant and antimicrobial properties of the lichens *Cladonia foliacea, Dermatocarporn miniatum, Evernia divaricata, Evernia prunastri* and *Neofuscelia pulla*. Pharmaceutical Biology, 44, 247-252.

Bilgin Sökmen, B. Kınalıoğlu, K., Aydın, S. (2018). Evaluation of antifungal potentials and antioxidant capacities of some foliose lichen species. Süleyman Demirel University Journal of Natural and Applied Sciences, 22, 148-152.

Candan, M., Yılmaz, M., Tay, T., Erdem, M., Özdemir Türk, A. (2007). Antimicrobial activity of extracts of the lichen *Parmelia sulcata* and its salazinic acid constituent. Zeitschrift für Naturforschung C, 62, 619-621.

CLSI (Clinical and Laboratory Standards Institute). (2008). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard. CLSI document M38-A2. Clin Lab Stand Inst (2nd ed.).

Goel, M., Dureja, P., Rani, A., Uniyal, P.L., Laatsch, H. (2011). Isolation, characterization and antifungal activity of major constituents of the Himalayan lichen *Parmelia reticulate* Tayl. Journal of Agricultural and Food Chemistry, 59, 2299-2307.

Güllüce, M., Aslan, A., Sokmen, M., Sahin, F., Adigüzel, A., Agar, G., Sokmen, A. (2006). Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis, Platismatia glauca, Ramalina pollinaria, Ramalina polymorpha* and *Umbilicaria nylanderiana*. Phytomedicine 13, 515-521.

Halama, P., Van Haluwin, C. (2004). Antifungal activity of lichen extracts and lichenic acids. BioControl, 49, 95-107.

Karabulut, G., Ozturk, S. (2015). Antifungal activity of *Evernia prunastri, Parmelia sulcata, Pseudevernia furfuracea* var. *furfuracea*. Pakistan Journal of Botany, 47, 1575-1579.

Kosanić, M., Manojlović, N., Janković, S., Stanojković, T. Ranković, B. (2013). *Evernia prunastri* and *Pseudoevernia furfuraceae* lichens and their major metabolites as antioxidant, antimicrobial and anticancer agents. Food and Chemical Toxicology, 53, 112-118.

Kosanić, M., Ranković, B. (2011). Antibacterial and antifungal activity of different lichens extracts and lichen acid. Research Journal of Biotechnology, 6, 23-26.

Mitrović, T., Stamenković, S., Cvetković, V., Tošić, S., Stanković, M., Radojević, I., Stefanović, O., Čomić, L., Dačić, D., Ćurčić, M., Marković, S. (2011). Antioxidant, antimicrobial and antiproliferative activities of five lichen species. International Journal of Molecular Sciences, 12, 5428-5448.

Ranković, B., Mišić, M., Sukdolak, S. (2007). Evaluation of antimicrobial activity of the lichens *Lasallia pustulata, Parmelia sulcata, Umbilicaria crustulosa* and *Umbilicaria cylindrica*. Microbiology, 76, 723-727.

Smith, C.W., Aptroot, A., Coppins, B.J., Fletcher, A., Gilbert, O.L., James, P.W., Wolseley, P.A. (2009). The Lichens of Great Britain and Ireland. 2nd ed. London: The British Lichen Society.

Song, P.P., Zhao, J., Liu, Z.L., Duan, Y.B., Hou, Y.P., Zhao, C.Q., Wu, M., Wei, M., Wang, N.H., Lv, Y., Han, Z.J. (2017). Evaluation of antifungal activities and structure-activity relationships of coumarin derivatives. Pest Management Science, 73, 94-101.

Türk, H., Yılmaz, M., Tay, T., Özdemir Türk, A., Kıvanç, M. (2006). Antimicrobial activity of extracts of chemical races of the Lichen *Pseudevernia furfuracea* and their physodic acid, chloroatranorin, atranorin, and olivetoric acid constituents. Zeitschrift für Naturforschung C, 61, 499-507.

Winn, W.C., Allen, S.D., Janda, W.M., Koneman, E.W., Procop, G.W., Schreckenberger, P.C., Woods, G.L. (2006). Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Baltimore and Philadelphia: Lippincott Williams and Wilkins.

Wirth, V., Hauck, M., Schultz, M. (2013). Die Flechten Deutschlands. Stuttgart: Ulmer.

Xu, M., Heidmarsson, S., Olafsdottir, E.S., Buonfiglio, R., Kogej, T., Omarsdottir, S. (2016). Secondary metabolites from cetrarioid lichens: Chemotaxonomy, biological activities and pharmaceutical potential. Phytomedicine, 23, 441-459.

Yılmaz Cankılıç, M., Yılmaz Sarıözlü, N., Candan, M., Tay, T. (2017). Screening of antibacterial, antituberculosis and antifungal effects of lichen *usnea florida* and ıts thamnolic acid constituents. Biomedical Research, 28, 3108-3113.

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