www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 10/1 (2017) 65-68

Research article/Araştırma makalesi

Antimycobacterial activity of some lichen extracts

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Abstract

The acetone, methanol and chloroform extracts of lichens *Cetraria aculeata*, *Cladonia foliacea*, *Parmelia sulcata*, *Pseudevernia furfuracea* var. *furfuracea*, *Ramalina farinacea* and *Tornabea scutellifera* were investigated for antimycobacteral properties against *Mycobacterium tuberculosis* H37Rv strain using Microplate Alamar Blue Assay (MABA). All of the lichen extracts showed considerable antimycobacterial effect with minimal inhibitory concentration (MIC) values ranging from 1250 to 156.25 µg/ml. Among the tested lichens, the chloroform extract of the lichen *Pseudevernia furfuracea* var. *furfuracea* exhibited the strongest activity with an MIC value of 156.25 µg/ml.

Key words: antimycobacterial activity, lichen, lichen extracts, MABA, *Mycobacterium tuberculosis*

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Bazı liken ekstraktlarının antimikobakteriyal aktivitesi

Özet

Cetraria aculeata, *Cladonia foliacea*, *Parmelia sulcata*, *Pseudevernia furfuracea* var. *furfuracea*, *Ramalina farinacea* ve *Tornabea scutellifera* likenlerinin aseton, metanol ve kloroform ekstraktlarının *Mycobacterium tuberculosis* H37Rv suşuna karşı antimikobakteriyal özellikleri Microplate Alamar Blue Assay (MABA) kullanılarak incelenmiştir. Liken ekstraktlarının tümü 1250-156.25 µg/ml minimal inhibisyon konsantrasyonu (MİK) aralığındaki değerler ile önemli ölçüde antimikobakteriyal etki göstermiştir. Test edilen likenler arasında *Pseudevernia furfuracea* var. *furfuracea* likeninin kloroform ekstraktı 156.25 µg/ml'lik MİK değeri ile en güçlü aktiviteyi göstermiştir.

Anahtar kelimeler: antimikobakteriyal aktivite, liken, liken ekstraktları, MABA, *Mycobacterium tuberculosis*

1. Introduction

Tuberculosis (TB) is one of the most important infectious diseases caused by mycobacteria belonging to the *Mycobacterium tuberculosis* complex that are a major cause of morbidity and mortality worldwide. According to the latest global tuberculosis report of World Health Organization (WHO), there were an estimated 10.4 million new TB cases (including 1.2 million among HIV-positive people) worldwide in 2015, of which 5.9 million were among men, 3.5 million among women and 1.0 million among children and 1.4 million TB deaths and also TB was one of the top 10 causes of death (WHO, 2016). Moreover in this report, there were an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100 000 people with rifampicin-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment in 2015 (WHO, 2016). The discovery and development of new antituberculosis compounds are needed because number of multi-drug resistant isolates of *M. tuberculosis* increases (Cantrell et al., 2001).

Natural products and their derivatives obtained from plants, bacteria, fungi, lichens, marine organisms and associated with their microorganisms etc. have showed antimycobacterial activity (König et al., 2000; Okunade et al., 2004; Pauli et al., 2005; Honda et al., 2010; Cheng et al., 2012; Nguta et al., 2016). These natural compounds have great importance in antibiotic drug discovery.

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 BioDiCon. 642-0117

Lichens are symbiotic associations between fungi and algae or cyanobacteria. These organisms for centuries have been used in many countries as a treatment for diseases of humans (Kosanić et al., 2012) such as *Cetraria islandica* (Iceland moss), *Lobaria pulmonaria* and *Cladonia* species were known as effective in the treatment of pulmonary tuberculosis (Vartia, 1973).

There are many investigations related to antimicrobial activities of lichens but there are limited studies in literature about the antimycobacterial activity of extracts and of pure substances isolated from lichens (Ingólfsdóttir et al., 1998; Honda et al., 2010; Lucarini et al., 2012). Therefore, the aim of the present study is to determine the activity of extracts of the lichens *Cetraria aculeata*, *Cladonia foliacea*, *Parmelia sulcata*, *Pseudevernia furfuracea* var. *furfuracea*, *Ramalina farinacea* and *Tornabea scutellifera* against *M. tuberculosis*.

2. Materials and methods

2.1. Lichen samples

The lichen species 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. The samples were identified by Dr. Mehmet Candan using standard keys (Smith et al., 2009; Wirth et al., 2013). The lichen samples were deposited in the Department of Biology, Herbarium of Anadolu University (ANES), Eskişehir, Turkey.

2.2. Preparation of the lichen extracts

Air-dried thalli of the lichen samples were powdered and 10 g portions were extracted in 100 ml of acetone, methanol and chloroform in ultrasonic bath for 30 min, then left at room temperature overnight. The lichen extracts were filtered using Whatman No. 1 filter paper and then concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at 4 °C until they were used for the analysis.

2.3. Antimycobacterial assay

Microplate Alamar Blue Assay (MABA) was used to determine antimycobacterial activities of the lichen extracts against *Mycobacterium tuberculosis* H37Rv ATCC 27294 (American Type Culture Collection). The dried lichen extracts were dissolved in 20% dimethylsulfoxide (DMSO) and then prepared dilution series from 10 mg/ml to 19.53 µg/ml using sterile distilled water. Rifampicin was used as standard at 0.8 mg/ml-1.5625 µg/ml concentration range. *M. tuberculosis* H37Rv was grown in ATCC® Medium 1395: Middlebrook 7H9 broth with ADC enrichment at 37 °C for 30 days. The turbidity of the cultures was adjusted to McFarland standard no 1. The incubation of all black, clear-bottomed, 96-well microtiter plates (Corning 3340, USA) were performed at the temperature of 37 °C for the period of 7 days. Then, a freshly prepared 1 : 1 mixture of Alamar Blue reagent (1 : 10 dilution, Invitrogen, 1025, USA) and 10% Tween 80 were added to each well on the 7th day of the incubation. The further incubation of the plates was performed at the temperature of 37 °C for the 24 h period. A color change from blue to pink indicated mycobacterial growth. The minimal inhibitory concentration (MIC) values were defined as the lowest concentration of the extracts that showed no color change. All experiments were carried out in triplicate.

3. Results

The potential antimycobacterial activity against *M. tuberculosis* H37Rv of acetone, methanol and chloroform extracts of the lichen species were shown in Table 1. The MIC values for the tested lichens extracts were determined within the range 1250-156.25 µg/ml. The chloroform extract of the lichen *Pseudevernia furfuracea* var. *furfuracea* showed the strongest antimycobacterial activity with 156.25 µg/ml MIC value among the screening lichens. The same MIC value (312.5 µg/ml) was obtained in all extract of lichen *Cladonia foliacea*. The chloroform extracts of the lichen *Cetraria aculeata* and *Pseudevernia furfuracea* var. *furfuracea* were showed stronger antimycobacterial activity than acetone and methanol extracts.

4. Conclusions and discussion

Previous studies were reported about antimicrobial activity of the lichens *Cetraria aculeata*, *Cladonia foliacea*, *Parmelia sulcata*, *Pseudevernia furfuracea* and *Ramalina farinacea* extracts and their constituents against different bacteria and yeasts (Tay et al., 2004; Yılmaz et al., 2004; Candan et al., 2007; Mitrović et al., 2011; Güvenç et al., 2012; Kosanić et al., 2013), but there are few studies related to antimycobacterial activity of these lichens extracts. Also, antimicrobial activity of the lichen *Tornabea scutellifera* has not been reported in literature.

Table 1. Minimal inhibitory concentration (MIC) values of the extracts of *C. aculeata*, *C. foliacea*, *P. sulcata*, *P. furfuracea*, *R. farinacea* and *T. scutellifera* against *M. tuberculosis* H37Rv

Lichens	**Lichen extracts**	**MIC (µg/ml)**
Cetraria aculeata	Acetone	1250
	Methanol	1250
	Chloroform	625
Cladonia foliacea	Acetone	312.5
	Methanol	312.5
	Chloroform	312.5
Parmelia sulcata	Acetone	625
	Methanol	625
	Chloroform	1250
Pseudevernia furfuracea var. *furfuracea*	Acetone	625
	Methanol	625
	Chloroform	156.25
Ramalina farinacea	Acetone	312.5
	Methanol	625
	Chloroform	312.5
Tornabea scutellifera	Acetone	625
	Methanol	1250
	Chloroform	625
Antibiotic (standard)		
Rifampicin		25

Ingólfsdóttir et al. (1998) screened the antimycobacterial activity of lichen metabolites usnic acid from *Cladonia arbuscula*, atranorin and lobaric acid from *Stereocaulon alpinum*, salazinic acid from *Parmelia saxatilis*, protolichesterinic acid from *Cetraria islandica* against *Mycobacterium aurum*, a non-pathogenic organism with a similar sensitivity profile to *M. tuberculosis*. They reported that usnic acid had the highest activity the with an MIC value of 32 µg/ml, while the atranorin, lobaric acid, salazinic acid and protolichesterinic acid had the activity with an MIC values of ≥125 µg/ml. A similar study was reported by Honda et al. (2010). They investigated of the activity against *M. tuberculosis* of twenty-six compounds from the lichens *Parmotrema dilatatum*, *Parmotrema tinctorum*, *Pseudoparmelia sphaerospora* and *Usnea subcavata*: depsides, depsidones and xanthones, usnic acid, derivatives from salazinic and lecanoric acids and lichexanthone. They determined diffractaic acid the most active compound among them with an MIC value 15.6 µg/ml followed by norstictic acid (MIC value 62.5 µg/ml) and usnic acid (MIC value 62.5 µg/ml). In that study, hypostictic acid with an MIC value 94.0 µg/ml and protocetraric acid with an MIC value 125 µg/ml showed moderate inhibitory activity while the other compounds showed lower inhibitory activity against *M. tuberculosis*, varying from 250 µM to1370 µM (MIC values ≥250 µg/ml). The activity of usnic acid against *M. tuberculosis* H37Rv as a 12.25 µg/ml was recorded by Ramos and Silva (2010). The acetone extract of lichen *Usnea steineri* was showed MIC values of 32 µg/ml against *M. tuberculosis* H37Rv while isolated compound usnic acid was even more effective against the same strain with MIC value of 8 µg/ml (Lucarini et al., 2012).

According to our results, the considerable antimycobacterial activity was observed with an MIC value of 156.25 µg/ml among the screening lichens extracts in our study**.** Further investigations to do with isolated compounds of the lichens in present study will be conducted more effective results against *M. tuberculosis*.

Acknowledgements

The authors wish to thanks Dr. Mehmet Candan (Anadolu University, Faculty of Sciences, Department of Biology) for identification of the lichens.

References

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

Cantrell, C.L., Franzblau, S.G., Fischer, N.H. (2001). Antimycobacterial plant terpenoids. Planta Medica, 67(8), 685-694.

Cheng, M.J., Wu, M.D., Yanai, H., Su, Y.S., Chen, I.S., Yuan, G.F., Hsieh, S.Y., Chen, J.J. (2012). Secondary metabolites from the endophytic fungus *Biscogniauxia formosana* and their antimycobacterial activity. Phytochemistry Letters, 5, 467-472.

Güvenç, A., Küpeli Akkol, E., Süntar, İ., Keleş, H., Yıldız S., Çalış, İ. (2012). Biological activities of *Pseudevernia furfuracea* (L.) Zopf extracts and isolation of the active compounds. Journal of Ethnopharmacology, 144, 726-734.

Honda, N.K., Pavan, F.R., Coelho, R.G., de Andrade Leite, S.R., Micheletti, A.C., Lopes, T.I.B., Misutsu, M.Y., Beatriz, A., Brum, R.I., Leite, C.Q.F. (2010). Antimycobacterial activity of lichen substances. Phytomedicine, 17, 328-332.

Ingólfsdóttir, K., Chung, G.A.C., Skúlason, V.G., Gissurarson, S.R., Vilhelmsdóttir, M. (1998). Antimycobacterial activity of lichen metabolites in vitro. European Journal of Pharmaceutical Sciences, 6, 141-144.

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.M, Ranković, B.R., Stanojković, T.P. (2012). Antioxidant, antimicrobial, and anticancer activities of three *Parmelia* species. Journal of the Science of Food and Agriculture, 92, 1909-1916.

König, G.M., Wright, A.D., Franzblau, S.G., (2000). Assessment of antimycobacterial activity of a series of mainly marine derived natural products. Planta Medica, 66(4), 337-342.

Lucarini, R., Tozatti, M.G., Salloum, A.I.O., Crotti, A.E.M., Silva, M.L.A., Gimenez, V.M.M., Groppo, M., Januário, A.H., Martins, C.H.G., Cunha W.R. (2012). Antimycobacterial activity of *Usnea steineri* and its major constituent (+)-usnic acid. African Journal of Biotechnology, 11(20), 4636-4639.

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.

Nguta, J.M., Appiah-Opong, R., Nyarko, A.K., Yeboah-Manu D., Addo, P.G.A., Otchere, I.D., Kissi-Twum, A. (2016). Antimycobacterial and cytotoxic activity of selected medicinal plant extracts. Journal of Ethnopharmacology, 182, 10-15.

Okunade, A.L., Elvin-Lewis, M.P., Lewis, W.H. (2004). Natural antimycobacterial metabolites: Current status. Phytochemistry, 65, 1017-1032.

Pauli, G.F., Case, R.J., Inui, T., Wang, Y., Cho, S., Fischer, N.H., Franzblau, S.G. (2005). New perspectives on natural products in TB drug research. Life Sciences, 78, 485-494.

Ramos D.F., Silva, P.E.A. (2010). Antimycobacterial activity of usnic acid against resistant and susceptible strains of *Mycobacterium tuberculosis* and non-tuberculous mycobacteria. Pharmaceutical Biology, 48(3), 260-263.

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.

Tay, T., Türk, A.Ö., Yılmaz, M., Türk, H., Kıvanç, M. (2004). Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstictic acid, and protocetraric acid constituents. Zeitschrift für Naturforschung, 59c, 384-388.

Vartia, K.O. 1973. Antibiotics in lichens. In: Ahmadjian, V., Hale, M.E. (Eds.), The Lichens. New York: Academic Press, pp. 547-561.

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

World Health Organization (WHO). (2016). Global tuberculosis report 2016. http://www.who.int (Erişim tarihi: 09.11.2016).

Yılmaz, M., Türk, A.Ö., Tay, T., Merih, K. (2004). The antimicrobial activity of extracts of the lichen *Cladonia foliacea* and its (-)-usnic acid, atranorin, and fumarprotocetraric acid constituents. Zeitschrift für Naturforschung, 59c, 249-254.

(Received for publication 25 January 2017; The date of publication 15 April 2017)