Düzce University Journal of Science & Technology

Research Article

Evaluation on Antimicrobial Activity of The Lichen *Pleurosticta acetabulum*

Basaran Dulger [a,*], Gorkem Dulger [b], Bahar Gedik [c], Seyhan Oran [d]

[a] *Department of Biology, Faculty of Science and Arts, Duzce University, 81620 Konuralp/Duzce, Turkiye*

[b] *Department of Medical Biology, Faculty of Medicine, Duzce University,81620 Konuralp/Duzce, Turkiye*

[c] *Department of Field Crops, Faculty of Agriculture, Duzce University,81620 Konuralp/Duzce, Turkiye*

[d] *Department of Biology, Faculty of Science and Arts, Uludag University,16059 Nilufer/Bursa, Turkiye*

** Sorumlu yazarın e-posta adresi: basarandulger@duzce.edu.tr*

DOI: 10.29130/dubited.1082155

ABSTRACT

In this study, the ethanol extract of the lichen *Pleurosticta acetabulum* (Neck.) Elix & Lumbsch (Parmeliaceae) was investigated for their antimicrobial activity against various microorganisms by the disc diffusion method. *Mycobacterium smegmatis* CCM 2067, *Micrococcus luteus* CCM 169, *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 15313, *Klebsiella pneumoniae* UC57, *Bacillus cereus* ATCC 7064, *Escherichia coli* ATCC 10536, *Rhodotorula rubra* DSM 70403, *Candida albicans* ATCC 10231 and *Kluyveromyces fragilis* ATCC 8608 were used as test microorgams. We have found that the ethanol extract of the lichen has a potential antimicrobial effect against all tested microorganisms, forming inhibition zones between 12.6-22.4 mm, as compared with the standard antibiotics. Notably, the extract has a strong effect against *Escherichia coli* and *Staphylococcus aureus*, especially *Candida albicans.* In conclusion, *P. acetabulum* may assist in the discovery of new antimicrobial agents that can serve as selective agents or in the preparation of new combined therapeutic drugs. However, the effect of this lichen species on more pathogenic organisms should be investigated and further and detailed pharmacological and toxicological studies should be conducted.

Keywords: *Pleurosticta acetabulum, The lichen, Antimicrobial activity*

Pleurosticta acetabulum Liken Türünün Antimikrobiyal Aktivitesi Üzerine Değerlendirme

ÖZ

Bu çalışmada, *Pleurosticta acetabulum* (Neck.) Elix & Lumbsch (Parmeliaceae) liken türünden elde edilen etanol ekstraktının antimikrobiyal aktivitesi, disk difüzyon yöntemi kullanılarak, *Mycobacterium smegmatis* CCM 2067, *Micrococcus luteus* CCM 169, *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 15313, *Klebsiella pneumoniae* UC57, *Bacillus cereus* ATCC 7064, *Escherichia coli* ATCC 10536, *Rhodotorula rubra* DSM 70403, *Candida albicans* ATCC 10231 ve *Kluyveromyces fragilis* ATCC 8608 test mikroorganizmalarına karşı araştırılmıştır. Standart antibiyotikleri ile karşılaştırıldığında, elde edilen etanol ekstraktının tüm test mikroorganizmalarına karşı (12.6-22.4 mm değerleri arasında) potansiyel bir aktivite gösterdiği belirlenmiştir. Sonuç olarak, *P. acetabulum* seçici ajan olarak hizmet edebilecek yeni antimikrobiyal ajanların keşfedilmesinde veya yeni konbine terapotik ilaçların hazırlanmasında yardımcı olabilir. Ancak bu liken türünün daha fazla mikroorganizmalar üzerindeki etkisinin araştırılması, daha ileri ve detaylı farmakolojik ve toksikolojik araştırmaların yapılması gerekmektedir.

Anahtar Kelimeler: *Pleurosticta acetabulum, Liken, Antimikrobiyal aktivite*

Received: 03/03/2022, Revised: 31/03/2022, Accepted: 05/04/2022

I. INTRODUCTION

It is known that lichens are beneficial for diseases such as heart, bronchitis, asthma, leprosy, blood, and scabies, so they have been mentioned quite a lot in the Ayurvedic treatment system. Also, they have long been used for bleeding, thirst, and vomiting [1].

Lichen secondary metabolites with antimicrobial, cytotoxic, antioxidant, anti-inflammatory and antipyretic properties are also potential sources of pharmaceutically beneficial chemicals [2].

As it known many lichen species have very interesting pharmacological and biological properties. However, there are few studies in the literature investigating the potential of *Pleurosticta acetabulum* (Neck.) Elix & Lumbsch (Parmeliaceae). For this reason, it is the aim of the present study to reveal the antimicrobial activity spectrum of this lichen species against various microorganisms.

II. MATERIALS AND METHODS

A. THE LICHEN MATERIAL

The samples of the lichen *Pleurosticta acetabulum* (Neck.) Elix & Lumbsch were collected from the mountain Uludağ, Soğukpınar, Bursa, Turkey in August, 2011 (40 03 44.7 N and 29 07 53.2 E) and identified by Dr. Seyhan Oran. A voucher sample was kept in author's personal collections (voucher number; BD.305-5)

B. PREPARATION OF EXTRACTS

Lichen samples collected from the field were dried at 40 °C (12 h) and ground into powder. Then, 20 g of this powdered lichen material was weighed and extracted. Extraction was carried out by Soxhlet with 150 mL of ethanol (95%) (24 h) [3]. The extract obtained after extraction was filtered through Whatman filter No.1, then evaporated at 55 °C using a rotary evaporator under vacuum (yield: 12.6% for ethanol). The extract was dissolved using DMSO to a final concentration of 1 g/mL for prescreening.

C. STRAINS

Mycobacterium smegmatis CCM 2067, *Micrococcus luteus* CCM 169, *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 15313, *Klebsiella pneumoniae* UC57, *Bacillus cereus* ATCC 7064, *Escherichia coli* ATCC 10536, *Rhodotorula rubra* DSM 70403, *Candida albicans* ATCC 10231 and *Kluyveromyces fragilis* ATCC 8608 were used as test microorganisms.

D. ANTIMICROBIAL ASSAY

The lichen extracts were dissolved in 10% aqueous DMSO (dimethyl sulfoxide). The final concentration was adjusted to 200 mg/mL. The extracts were then passed through a 0.45 mm membrane filter for sterilization. Empty discs (6 mm in diam.) were impregnated with 50 mL of this extract, each at a concentration of 200 mg/mL. Bacterial cultures to be used in the study were inoculated into Nutrient Broth (Difco) and incubated at 35±0.1 °C for 24 h. In addition, the yeast cultures to be used were incubated in Malt Extract Broth (Difco) for 48 h at 25±0.1 °C. An inoculum containing 10^6 bacterial cells was placed on Mueller-Hinton Agar (Oxoid) plates [4]. MH-GMB (2% glucose and methylene blue 0.5 μg/ml) agar plates were prepared for yeast. 10^8 yeast cells/mL were inoculated on MH-GMB plates [5].

The discs injected with the lichen extracts were placed. Then, plates were then incubated at 4°C for 2 h. After this period, the plates in which the yeast cultures were cultivated were incubated at 25±0.1°C for 48 h, and those cultured with bacterial cultures for 24 h at 35±0.1°C [4-6]. Finally, the diameters of the zones of inhibition that occurred on the media were measured and using a physical ruler such as a meter scale (as millimeters). Experiments in the study were performed in triplicate and evaluated. For comparison, a suitable reference antimicrobial agent disc was placed on the medium, taking into account the characteristics of the test microorganisms.

III. RESULTS AND DISCUSSION

Table 1 is indicated that the zones of inhibition formed by the lichen extracts assayed against tested microorganisms and the standard comparison antibiotics.

As clearly indicated in Table 1, lichen extracts have a potential antibacterial effect against all tested bacteria and formed zones of inhibition between 12.6-21.2 mm. The ethanol extract has much greater antibacterial zones than those of all comparison antibacterial agents except for Oflaxacin (5 mg) and tetracycline (30 mg) against *Escherichia coli*. Compared to the standard comparison antibiotics Cefotaxime (30 mg), Ampicillin 10 and Penicillin G (10 units) *Staphylococcus aureus* is more susceptible. Effect of the extracts against *Klebsiella pneumoniae* and *Micrococcus luteus* is far below the activities of all comparison antibiotics used in this study. The extract has a stronger activity than those of only one the standard antibiotic, Cefotaxime (30 mg) and Penicillin G (10 units) against *Mycobacterium smegmatis* as acid-fast bacterium and *Proteus vulgaris*, respectively. Against *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Bacillus cereus*, the extract has shown a moderate activity according to the effects of some standard comparison antibiotics. When the antifungal activity results of the extract are examined, against all the yeast cultures, the extract has shown more strong effect in comparison with Nystatin standard, forming zones of 20.8-24.4 mm. The strongest effect was detected against *Candida albicans* (24.4 mm).

Antibiotic drugs needed for the treatment of microbial infections and diseases from plant sources in obtaining has been used since. Also, many lichens with potential in this respect. its importance has been increasing recently. Many of the lichen species showed very interesting findings of biological activity. However, only one study in the literature has investigated the potential of *Pleurosticta acetabulum* [7]. In that study, atranorin, norstictic, salazinic evernic acid and protocetraric were identified as compounds of *P. acetabulum*. The acetone extract obtained from the lichen was investigated for their antimicrobial activities against various bacteria and fungi using broth microdilution method. MIC values of the lichen acetone extract were found to range between 5-20 mg/mL for fungal cultures and 1.25-20 mg/mL for bacterial cultures. It was reported that the extract had no effect against *E. coli* and *Aspergillus flavus*, which were found to be the most resistant bacterial and fungal cultures. The potential antimicrobial activities of evernic and norstitic acids have been previously reported in the literature [7]. Both compounds were tested for their antibacterial activities against Gram positive and Gram negative bacteria, and as a result, Gram-negative bacteria were determined to be more resistant to these compounds by this study. Manojlovic et al. reported the antimicrobial effect of salazinic and protocetraric acids [8]. They also reported that it showed strong antimicrobial activity for atranorin [9-10]. Our findings clearly revealed that *P. acetabulum* has potent antimicrobial effects against the tested microorganisms. Notably, antimicrobial activity is found to be strongly effective against *Staphylococcus aureus* and *Escherichia coli* among the tested bacteria, against all yeast cultures especially opportunistic skin pathogen, *Candida albicans*. These compounds mentioned above may be responsible for their antimicrobial effects against microorganisms. These results indicated that the lichen *P. acetabulum* may be a good candidate as an inhibitor for *Escherichia coli* and *Staphylococcus aureus*, especially *Candida albicans*.

It is seen that the antimicrobial activities of lichen species vary according to the area of collection and the differences in the solvents and methods used while obtaining lichen extracts. It is thought that parameters such as humidity and air pollution rates of the regions where lichens are found, air temperature and light differences have a role in the synthesis of secondary metabolites, and as a result, antimicrobial activities vary. However, the solvents used in obtaining lichen extracts also affect the antimicrobial activities of lichens [11]. Therefore, more similar studies more comprehensive data will be available.

Table 1. *Summary of antimicrobial activity of P. acetabulum extracts and standard comparison antibiotics.*

Microorganisms	**The ethanol extract** *(50 mg/mL)*	**Standard antibiotics/inhibition zones** *(mm)*[a]						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Escherichia coli	21.2	18.2	12.6	10.6	20.4	24.2	26.2	Nt
Staphylococcus aureus	15.2	13.6	15.8	12.6	12.8	20.8	21.2	Nt
Klebsiella pneumoniae	13.8	18.4	15.2	14.2	21.2	24.6	20.6	Nt
Pseudomonas aeruginosa	12.6	10.2	11.2	28.6	10.8	28.2	24.6	Nt
Proteus vulgaris	12.8	10.4	17.2	18.2	20.2	22.4	21.8	Nt
Bacillus cereus	14.6	15.4	12.8	13.4	16.8	26.4	19.8	Nt
Mycobacterium smegmatis	13.8	14.8	20.2	11.6	18.6	24.8	22.2	Nt
Listeria monocytogenes	14.2	11.2	12.6	17.2	24.2	28.6	19.8	Nt
Micrococcus luteus	16.8	28.4	26.4	24.8	26.2	24.2	20.2	Nt
Candida albicans	24.4	Nt	Nt	Nt	Nt	Nt	Nt	20.4
Kluyveromyces fragilis	20.8	Nt	Nt	Nt	Nt	Nt	Nt	18.2
Rhodotorula rubra	21.6	Nt	Nt	Nt	Nt	Nt	Nt	18.6
Ethanol (control)	0	0	0	0	0	0	0	0

[a] Includes diameter of disk (6 mm)

1: P10, Penicillin G (10 units); 2: SAM20, Ampicillin 10 mg; 3: CTX30,Cefotaxime 30 mg; 4: V30, Vancomycin 30 mg; 5: OFX 5, Oflaxacin 5 mg; 6: TE30, Tetracycline 30 mg; 7: NY100, Nystatin 100 mg

Nt: Not tested

IV. CONCLUSION

In this study, by revealing the broad spectrum of antimicrobial activity by *P. acetabulum*, it may help the discovery of new antibiotic agents that can serve as selective agents in the treatment and prevention of disease, as well as shed light on all other researches aimed at increasing the proliferation and quality of this study. The effect of the lichen on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

V. REFERENCES

[1] V. Shukla, G.P. Joshi and M.S.M. Rawat, "Lichens as a potential natural source of bioactive compounds: a review," *Phytochem Rev*., vol. 9, no. 2, pp. 303-314, 2010.

[2] B. Rankovic and M. Kosanic, "Lichen as a potential source of bioactive secondary metabolites. Lichen secondary metabolites," *Springer International Publishing Switzerland*, pp. 1-26, 2015.

[3] N.H. Khan NH, M.S.A. nur-E Kamal and M. Rahman, "Antibacterial activity of *Euphorbia thymifolia* Linn," *Indian J Med Res*., vol. 87, pp. 395-397, 1988.

[4] CLSI. Performance standards for antimicrobial disk susceptibility tests, approved standard-eleventh edition. CLSI document M02-A11, vol. 32, no. 1, Clinical and Laboratory Standards Institute, Wayne, PA, 2012.

[5] NCCLS. Method for antifungal disk diffusion susceptibility testing of yeasts: proposed guideline M44-P. National Committee for Clinical Laboratory Standards, Wayne, PA, 2003.

[6] C.H. Collins, P.M. Lyne and G.M. Grange, "Collins and Lyne Microbiological methods," 6th ed., London, UK: Butterworth, 1989.

[7] J. Tomovic, M., Kosanic, S. Ristic, B. Rankoviv, T. Stanojkovic and N. Monojlovic, "Chemical composition and bioactive properties of the lichen, *Pleurosticta acetabulum*," *Trop J Pharm Res*., vol. 16, no. 12, pp. 2977-2984, 2017.

[8] N. Manojlovic, B. Rankovic, M. Kosanic, P. Vasiljevic and T. Stanojkovic, "Chemical composition of three *Parmelia* lichens and antioxidant, antimicrobial and cytotoxic activities of some their major metabolites," *Phytomed,* vol. 19, no. 13, pp. 1116-1172, 2012.

[9] Y. Yilmaz, A.O. Turk, T. Tay and M. Kivanc, "The antimicrobial activity of extracts of the lichen *Cladonia foliaceae* and its (-)-usnic acid, atronin and fumarprotocetraric acid constituents," *Z Naturforsch C,* vol. 59, no. 3-4, pp. 249-254, 2004.

[10] V.M. Thadhani, I.M. Choudhary, S. Khan and V. Karunaratne, "Antimicrobial and toxicolojical activities of some depsides and depsidones," *J Natl Sci Found,* vol. 40, pp. 43-48, 2012.

[11] E. Ucarkus, B. Gokalsin, N. Yildirim, B. Acikgoz, G. Ozyigitoglu and N.C. Sesal, "Antibacterial activities of extracts of lichen samples collected from Balikesir region on *Escherichia coli* ATCC 25922," *Journal of Turkish Society of Microbiology*, vol. 47, pp. 26-32, 2017.