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Antimicrobial Potential of *Usnea longissima Ach.* Lichen Against Human Pathogens

Usnea longissima Ach. Likeninin İnsan Patojenlerine Karşı Antimikrobiyal Potansiyeli

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Abstract
Lichens are a symbiotic associations between a fungus and algae and/or cyanobacteria. Lichens use as food and dye source, air pollution indicator, medicinal and decoration. In this study, antimicrobial activity of ethyl acetate and ethanol extracts of *Usnea longissima* Ach. lichen was searched by disc diffusion method. While inhibition zones range from 14.5 mm to 24.5 mm for bacteria, inhibition zones range from 10 mm to 32 mm for fungi. Lichen extracts exhibited higher activity than gentamicin and tetracycline but they showed similar activity to nystatin. The MIC values of the extracts varied between 2-117 µg/mL against bacteria; while the MIC values of the extracts varied between 4-59 µg/mL against fungi. According to the obtained results, it could be said that *U. longissima* might be an alternative to synthetic antimicrobial agents.

Keywords: Antimicrobial activity, Lichen, Microorganism

Öz
Likenler bir mantar ve bir alg ve/ya da siyanobakteri arasındaki simbiyotik birlikteliklerdir. Likenler besin ve boya kaynağı, hava kirliliği indikatörü, tıbbi ve dekorasyon amaçlı kullanılmaktadır. Bu çalışmada, Usnea longissima Ach. likeninin etanol ve etil asetat ekstraktlarının antimikrobiyal aktiviteleri disk difüzyon metodu ile araştırıldı. Bakteriler için inhibisyon zonları 14.5 mm ve 24.5 mm arasında değişirken; mantarlar için 10 mm ve 32 mm arasında değişmektedir. Liken ekstraktları gentamisin ve tetrasiklinden daha yüksek ve nistatine benzer aktivite göstermiştir. Ekstraktların bakterilere karşı MİK değerleri 2-117 µg/mL arasında değişirken; mantarlara karşı MİK değerleri 4-59 µg/mL arasında değişmektedir. Elde edilen sonuçlara göre, U. longissima'nın sentetik antimikrobiyal ajanlara alternatif olabileceği söylenebilir.

Anahtar kelimeler: *Antimikrobiyal aktivite, Liken, Mikroorganizma*

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1. Intrdouction

Antibiotics provide the first therapy of microbial infections. When the discovery of these medicines as chemotherapeutic agents it was thought that this would guide to wipe out spreading infectious diseases. Recently, most of the microorganisms gained resistance current antibiotics. Therefore, there is a perpetual and urgent requirement to find brand antimicrobial compounds which possess various chemical structures and new mechanisms of action (Amenu, 2014).

Medicinal plants symbolize natural alternatives to synthetic antimicrobial agents. Plants are utilized medicinally in worldwide. A wide range of medicinal plant is utilized for extraction as raw drugs. Moreover, medicinal plants are trusted to be significant source of brand chemical substances with potential therapeutic actions. The secondary compounds of plants might be utilized as intermediates for the production of brand drugs (Chandra, 2013).

Lichen is a symbiotic association between a fungus and algae and/or cyanobacteria. They are used as food and dye source, air pollution indicator, medicinal and decoration. *Usnea longissima* is one of the medical lichens used in the treatment of bone fractures and sprains, in the treatment of leg injuries and ulcers (Atalay et al., 2011). Moreover, *U. longissima* is used as an expectorant, wound dressing material and nose bleed in various parts of the world (Ağar et al., 2011). *U. longissima* is currently used for the treatment of tuberculous lymphadenitis (Hobbs, 1990). *U. longissima* has antiulcerogenic effect on stomach ulcer model induced by indomethazine (Halici et al., 2005). *U. longissima* was used as an expectorant and a powder application to treat external ulcers in the name "Sun-Lo" by the Chinese. Moreover, it is also a major ingredient of Chinese medicine. In the Bolivian Andes, *U. longissima* is used as a medicine to heal cough and hoarseness. It has been used for stimulating menstruation or induce abortion by Unani medicine (Prateeksha et al., 2016).

It was aimed to investigate antimicrobial potential of ethyl acetate and ethanol extracts of *U. longissima* against *Salmonella enterica* serovar *typhimurium* ATCC 14028, *Proteus vulgaris* FMC 1, *Enterobacter aerogenes* CCM 2531, *Yersinia pseudotuberculosis* (laboratory isolate), *Escherichia coli* ATCC 35218, *Gordonia rubripertincta* (laboratory isolate), *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* IMG 22, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 ROMA, *Saccharomyces cerevisiae, Candida tropicalis* ATCC 13803 and *Candida albicans* FMC 17.

2. Material and Methods

2.1. Collection and Identification of Lichen Material

Lichen samples (Figure 1) were collected from the trunks of coniferous tree from Dereli district in the province of Giresun in 2017. Collected lichen specimens was dried at room temperature and identified as *Usnea longissima* Ach. according to the Brodo et al. (2001). The voucher samples deposited in the herbarium of the Biology Department, Faculty of Science and Arts, Giresun University, Giresun, Turkey).

Figure 1. *Usnea longissima* Ach.

2.2. Lichen Extraction

Soxhlet apparatus was used to obtain of lichen extracts. 25 g of the powdered sample was extracted with 250 mL ethyl acetate and ethanol solvents, separately. The extraction process followed by filtration with Whatman filter paper no 1. Then, the filtered extract concentrated in vacuum at 40 °C using a rotary evaporator (Kumar et al., 2012).

2.3. Microorganisms

5 Gram (+), 5 Gram (-) bacteria and 3 fungi were used in this study. *Salmonella enterica* serovar *typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923 and *Saccharomyces cerevisiae* were provided by Giresun Province Control Laboratory. *Proteus vulgaris* FMC 1, *Enterobacter aerogenes* CCM 2531, *Bacillus subtilis* IMG 22, *Candida tropicalis* ATCC 13803 and *Candida albicans* FMC 17 were provided by Fırat University. *Escherichia coli* ATCC 35218 was provided by Giresun University. *Yersinia pseudotuberculosis* (laboratory isolate) and *Gordonia rubripertincta* (laboratory isolate) were provided by Yeditepe University. *Enterococcus faecalis* ATCC 29212 and *Bacillus cereus* 702 ROMA were provided by Recep Tayyip Erdoğan University.

2.4. Antimicrobial Activity

The antimicrobial potential of the extracts of *U. longissima* was identified by disc diffusion assay. Each lichen extract was dissolved in dimethyl sulfoxide (DMSO) (2.5% concentration) at 30 mg/mL concentration. Dissolved extracts were sterilized through 0.45 µm pore sized filter. Gentamicin and tetracycline were used as standard antimicrobial agent. The turbidity of bacterial suspensions was adjusted 0.5 Mc Farland standards (10^8 CFU/mL), then, the bacterial suspension inoculated into MHA plates and allowed to dry. Discs (5 mm diameter) were put onto the inoculated agar. 25 µL ethyl acetate extract of *U. longissima*, 25 µL ethanol extract of *U. longissima* and 25 µL DMSO were added to discs, separately. The inoculated plates were left in refrigerator for one hour then plates were incubated at 37 °C overnight. Diameters of zones were measured. The sensitivity of the microorganisms to the studied lichens was revealed by measuring the inhibitory zones size on the agar surface around the discs (Murray et al., 1995; Sarić et al., 2009).

Antifungal activity was determined by disc diffusion method with Sabaroud Dextrose Agar (SDA) and Sabaroud Dextrose Broth (SDB). The procedure which was used in bacteria was used to determine antifungal activity except for the turbidity of fungal suspensions were adjusted 0.5 Mc Farland standard (10^7 CFU/mL) (Ertürk, 2006). Nystatin was used as standard antifungal agent. Discs were put (5 mm diameter) onto the agar and 25 µL ethyl acetate extract of *U. longissima*, 25 µL ethanol extract of *U. longissima* and 25 µL DMSO were added to discs, separetely. Plates were incubated at 35 °C for 48 h. Diameter of inhibition zones were measured in millimeters (Ünal et al., 2008). All the antimicrobial tests were carried twice.

The macrobroth dilution method was used to determine MIC values. Firstly, the 96 well plates were prepared by dispensing into each well 95 µL of Mueller Hinton Broth and 5 µL of the inoculums. 100 µL (prepared at 30 mg/mL concentration) ethyl acetate and ethanol extracts of *U. longissima* initially prepared at the concentration of 1 mg mL^{-1} was added into the first wells. Then, 100 µL from their serial dilutions were added into seven consecutive wells. This 96 well plate was incubated at 37 °C for bacteria overnight and 35 °C for 48 h for fungi. The MIC was expressed as the lowest concentration of the test compounds to inhibit the growth of microorganisms (Güllüce et al., 2004).

3. Results and Discussion

Plants are advantageous because of containing various compounds with healthy effects. Both essential oils of these plants and their extracts are useful in the treatment of many infectious diseases in the respiratory and gastrointestinal system, urinary tract. Because of the negative effects of antibiotics, increased resistance of antibiotics and the high production cost of generation of chemical compounds, drug companies are searching for brand alternatives. Some scientists stated that medicinal plants might be utilized in pharmaceutical industry. Rising awareness of people towards natural food and natural therapies seems to an alternative (Keskin et al., 2010).

The current study was performed to gain preliminary knowledge on the antimicrobial activity of *U. longissima*. Antimicrobial activity of the lichen extracts were determined by Kirby-Bauer technique of disc diffusion method. Minimum inhibitory concentration was taken out by Broth micro dilution method according to the

NCCLS guidelines. Antimicrobial activity of the tested lichen extracts was demonstrated in Table 1. Both ethanol and ethyl acetate extracts of *U. longissima* exhibited no activity against *Y. pseudotuberculosis*, *E. coli* and *S. enterica* serovar *typhimurium*. The extracts showed higher effect than the commercial antibiotic tetramycine and gentamicin, although extracts showed similar activity with standard antifungal agent nystatin. While the inhibition zones of the test bacteria were found ranged from 14.5 mm to 24.5 mm, the inhibition zones were detected ranged from 10 mm to 32 mm for fungi. Generally, gram (-) bacteria were more resistant than gram (+) bacteria against the lichen extracts. This situation might be arisen from the difference of cell wall in gram (+) and gram (-) bacteria.

Table 1. Inhibition zones of the tested extracts (mm)

Microorganism	EU	EAU	DMSO	Tetra	Genta	Nys
S. aureus	14.5	16	NA	17.5	19	NA
B. subtilis	23	21	NA	13	16	NA
Y. pseudotuberculosis	NA	NA	NA	NA	19	NA
E. aerogenes	21	22.5	NA	9.5	15	NA
B. cereus	25	21.5	NA	13	17.5	NA
P. vulgaris	23.5	24.5	NA	12.5	17	NA
E. faecalis	21	20.5	NA	NA	16.5	NA
E. coli	NA	NA	NA	14.5	17.5	NA
S. enterica serovar *typhimurium*	NA	NA	NA	14	17	NA
G. rubripertincta	17	18	NA	16	22	NA
C. tropicalis	27	25	NA	NA	NA	30
C. albicans	32	29.5	NA	NA	NA	30
S. cerevisiae	13	10	NA	NA	NA	13

EU: Ethanol extract of *U. longissima*; EAU: Ethyl acetate extract of *U. longissima*; NA: No Activity; Tetra: Tetracycline (10 µg/disc); Gen: Gentamicin (10 µg/disc); Nys: Nystatin (100 µg/disc)

Table 2 represents MIC values of the test extracts. The MIC values of the extract varied between 2-117 µg/mL against bacteria; while The MIC values of the extract varied between 4-59 µg/mL against fungi. Lichen extracts exhibited higher activity against fungi than bacteria.

Table 2. Results of MIC values of the extracts (µg/ml)

Microorganism	EU	EAU	Microorganism	EU	EAU
S. aureus	59	117	*E. faecalis*	29	59
B. subtilis	2	2	*G. rubripertincta*	59	117
E. aerogenes	7	14	*C. tropicalis*	7	4
B. cereus	14	29	*C. albicans*	59	7
P. vulgaris	14	7	*S. cerevisiae*	59	29

Similar reports on antimicrobial effects of *U. longissima* are available in the literatüre. For example, Maulidiyah et al. (2016) investigated chloroform fraction of *U. longissima* and it concluded that there was activity against *E. coli*, *S. aureus* and *S. typhi*. In contrast of this study, we found antimicrobial activity of ethanol and ethyl acetate extracts of *U. longissima* against *E. coli* and *S. typhimurium*. This difference could be related with collecting lichen samples from different locations and using different extract amounts and types. Kamal et al. (2015) searched antibacterial activity of ethanol and methanol extracts of *Usnea* sp. which collected from India against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi* and *E. coli*. They found activity in both ethanol and methanol extracts of *Usnea* sp. against *S. aureus*, *E. coli* and *S. thyphi*. However, in our study we found no activity against *E. coli* and *S. enterica* serovar *typhimurium* but we detected activity against *S. aureus*. This situation might be arisen from collecting lichens in different locations. Rauf et al. (2011) stated that hydroalcoholic and ethanolic extracts of *U. longissima* possess significant antibacterial property towards *S. aureus*, *B. cereus*, *E. coli* and *P. vulgaris*. In our survey, we also found activity for *S. aureus*, *B. cereus* and *P. vulgaris*; but no activity against *E. coli*. Dandapat and Paul (2015) investigated antimicrobial activity of *Usnea*

longissima against *E. coli*, *S. aureus* and *S. typhi*. Siddiqi et al. (2018) searched antimicrobial activity of silver nanoparticles using aqueous-ethanolic extract of *Usnea longissima*. Thippeswamy et al. (2011) revealed that ethanolic extract of *U. longissima* had activity against *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhimurium*, *E. coli*, *Trichoderma viridi* and *C. albicans*. Cansaran et al. (2006) determined that acetone extract of *U. longissima* had activity against *B. subtilis* and *B. megaterium* but had no activity against *E. coli*, *E. faecalis*, *P. mirabilis*, *S. aureus* and *P. aeruginosa*. In contrast to this study, we found activity against *S. aureus*, *B. subtilis* and *E. faecalis*. In this study, we found no activity against *E. coli*. Koçer et al. (2014) searched antimicrobial activity of hydroxyphenylimino ligands and their metal complexes of usnic acid isolated from *U. longissima*. It was concluded that the ligands and their complexes of the ligands exhibited between 11 mm to 32 mm inhibition zones against test microorganisms. The power of antimicrobial action depends on extract type, concentration of used extract and the tested microorganisms (Srivastava et al., 2013).

4. Conclusions

The obtained results revealed that ethyl acetate and ethanol extracts of *U. longissima* possess important antimicrobial activity against the tested bacteria and fungi which have significance in human therapy, animal and plant diseases. Further studies about antimicrobial activity as well as the isolation of the metabolites from the *U. longissima* lichen are needed. Therefore, the antimicrobial effect of lichen tested might be explained with new and broad studies by utilizing various solvents for extraction.

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