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Investigation of Antibacterial and Antifungal Effects of *Pediastrum boryanum* (Turpin) Meneghini

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Abstract

In this study, the antibacterial and antifungal effects of tris-HCL, n-butanol and ethanol extracts of *Pediastrum boryanum* (Turpin) Meneghini (Synonym *Pseudopediastrum boryanum* (Turpin) E. Hegewald), cultured from benthic and pelagic habitats of the Yeşilırmak River, on various microorganism species were investigated. Antimicrobial activity tests were performed on the microorganisms *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O 157:H7, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, *Candida albicans* ATCC 10239 by disk diffusion method. 0.5 M tris-HCL pH: 8.00, n-Butanol and ethanol were used as solvents. As a result, in antibacterial activity tests, it was determined that the buffer extract *of P. boryanum* had highly effective antibacterial properties on *Listeria monocytogenes* ATCC 7644. It was recorded that *Escherichia coli* O 157:H7 strain was the most susceptible microorganism, while *Bacillus subtilis* ATCC 6633 strain was the most resistant microorganism; the most effective solvent was 0.5 M Tris-HCL pH: 8.00. In antifungal activity tests, it was seen that the ethanol extract of *P. boryanum* had highly effective antifungal properties on *Candida albicans* ATCC 10239.

Keywords: *Pediastrum boryanum*, *Pseudopediastrum boryanum*, antibacterial activity, antifungal activity, disk diffusion method

Pediastrum boryanum (Turpin) Meneghini'nin Antibakteriyel ve Antifungal Etkilerinin Araştırılması

Öz

Bu çalışmada, Yeşilırmak Nehri'nin bentik ve pelajik habitatlarından alınarak kültürü yapılan *Pediastrum boryanum* (Turpin) Meneghini (Sinonimi *Pseudopediastrum boryanum* (Turpin) E.Hegewald)'un tris-HCL, n-butanol ve etanol ekstraktlarının çeşitli mikroorganizma türleri üzerindeki antibakteriyel ve antifungal etkileri araştırılmıştır. Antimikrobiyal aktivite testleri, disk difüzyon yöntemi kullanılarak *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O 157:H7, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, *Candida albicans* ATCC 10239 mikroorganizmaları üzerinde denenmiştir. Çözücü olarak 0.5 M Tris-HCL pH: 8.00, n-Butanol ve ethanol kullanılmıştır. Sonuç olarak, antibakteriyel aktivite testlerinde *P. boryanum*'un tampon ekstraktının *Listeria monocytogenes* ATCC 7644 üzerinde yüksek derecede antibakteriyel etkiye sahip olduğu belirlenmiştir. Ayrıca, *Escherichia coli* O157:H7 suşunun en duyarlı, *Bacillus subtilis* ATCC 6633 suşunun ise en dirençli mikroorganizma olduğu kaydedilmiş; en etkili çözücünün ise 0,5 M Tris-HCl (pH 8,00) olduğu tespit edilmiştir. Antifungal aktivite testlerinde ise *P. boryanum*'un etanol ekstraktının *Candida albicans* ATCC 10239 üzerinde yüksek derecede antifungal etkiye sahip olduğu görülmüştür.

Anahtar Kelimeler: *Pediastrum boryanum*, *Pseudopediastrum boryanum*, antibakteriyel aktivite, antifungal aktivite, disk difüzyon metodu

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1. Giriş

Algae are natural sources of high biological activity, containing numerous bioactive components. It is known that algae are rich in protein, amino acids, vitamins and various minerals, and contain polysaccharides, sterols and fatty acids, and therefore have a wide range of uses (El-Sheekh et al., 2006). At the same time, it contains secondary metabolites such as alkaloids, phenolic compounds, steroids and terpenoids, which have ecological, toxicological and pharmacological importance (Maschek and Baker, 2008; Somnath Chakraborty and Upasana Ghosh, 2010).

The increasing interest in algae is due to their wide biodiversity and specific pharmaceutically valuable molecules (Sousa, 2017).

Recently, the resistance of microorganisms to antibiotics and the remarkable presence of side effects in artificial drugs that are not seen or seen less in natural drugs have led scientists to research natural drugs and the public to use medicinal plants (Koçer and Sugeçti, 2015).

Algae have a wide variety of secondary metabolites that exhibit different biological activities such as anticancer, antifungal, anti-inflammatory and antioxidant activity. Due to these properties, they are considered as a source of bioactive compounds used in cosmetic formulations (Gnanavel et al., 2019).

Algae are also used in heavy metal poisoning, balancing the immune system, reducing high fever, regulating blood circulation, injuries, skin regeneration, lowering cholesterol and eliminating vascular occlusions (Meenakshi et al., 2011).

Algae have been used in traditional medicine by humans for many years; the bacteriostatic and bactericidal activity of some algal compounds against bacteria has been investigated by many researchers (Fitton, 2006; Salvador et al., 2007).

2. Material and Methods

2.1 Isolation and Culture of Algae

P. boryanum (Turpin) Meneghini was isolated in the laboratory by mechanical isolation and micro-injection methods after being collected from benthic and pelagic habitats of Yeşilırmak River (Tokat). The sampling point from Yeşilırmak is located in the city center of Tokat, at latitude 40˚ 21' 33.88" N and longitude 36˚38' 37.59" E. The samples were taken during the summer. Then, it was cultured with Allen, BG11 medium in a Sanyo MLR 351 brand climate cabinet at 26 ° C (155 µmol / m^2 /hour, 12:12 L:D period). After the pure cultured algae species were harvested, they were stored in the culture collection at -86 °C in a Sanyo ultra-low temperature freezer to be used in antibacterial and antifungal studies. The composition of the BG11 medium is shown in Table 1 (Rippka et al., 1979; Lobban et al., 1988).

2.2 Antibacterial and Antifungal Activity

The algal species were taken out from the -86 °C freezer and crushed with liquid nitrogen using a porcelain mortar. Then, solvents were added to them to form extracts (Moniharapon and Hashinaga, 2004; Sharma et al., 2004) and then prepared for activity tests.

2.3 Types of Microorganisms

In order to investigate the antimicrobial activities of the obtained extracts, 7 different test microorganisms were selected. The reference bacterial isolates used in this study were classified according to the Gram staining method. *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Listeria monocytogenes* ATCC 7644 were identified as Gram-positive, while *Escherichia coli* O157:H7, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhimurium* CCM 5445 were classified as Gram-negative bacteria. *Candida albicans* ATCC 10239, being a yeast, was not categorized by Gram staining and was included as a fungal reference. The prepared microorganism suspension was compared with the previously prepared 0.5 McFarland standard, and the turbidity of the suspension was homogenized using a UNICO brand UV-visible spectrophotometer (Şenol et al., 2007).

Table 1. BG11 medium composition

Component	Amount (mg/L)
$NaNO_3$	1500
$K_2HPO_4 \cdot 3H_2O$	40
$MgSO_4 \cdot 7H_2O$	75
$CaCl_2 \cdot 2H_2O$	36
Citric acid	6
Ferric ammonium citrate	6
EDTA (disodium salt)	1
Na_2CO_3	20
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.222
$Na_2MoO_4 \cdot 2H_2O$	0.39
$CuSO_4 \cdot 5H_2O$	0.079
$Co(NO3)_2 \cdot 6H_2O$	0.0494

2.4 Antibacterial and antifungal activity tests by disk diffusion method

The disk diffusion test was performed to evaluate the antimicrobial susceptibility of bacteria and fungi. Mueller-Hinton agar for bacterial cultures and Sabouraud dextrose agar for *Candida albicans* were obtained from Merck (Darmstadt, Germany). The antibiotic disks (penicillin G, ampicillin, gentamicin, chloramphenicol, streptomycin) and the antifungal disks (nystatin) were supplied by Oxoid (Hampshire, UK). Tris-HCl buffer (0.5 M, pH 8.0), n-butanol, and ethanol used for extract preparation were purchased from Sigma-Aldrich (St. Louis, MO, USA). Microbial suspensions were evenly spread over the agar surface using a sterile swab. After disk placement, bacteria were incubated at 35–37 °C for 18–24 hours, while *Candida albicans* was incubated at 30 °C for 24–48 hours. Following incubation, the inhibition zones were measured in millimeters (mm), and the susceptibility of the microorganisms was determined (Silici and Koç, 2006; Reller et al., 2009; CLSI, 2025; EUCAST, 2025).

2.5 Control group

Standard antibiotic disks were used as the positive control group and solvent (0.5 M Tris-HCL pH: 8.00, n-Butanol, Ethanol) impregnated disks were used as the negative control group. The standard concentrations of antibiotics used in disk diffusion tests were determined according to the CLSI and EUCAST guidelines. Penicillin G (CLSI 10 units / EUCAST 1 unit), ampicillin (CLSI 10 µg / EUCAST 2 µg), gentamicin (standard 10 µg, high-dose control 120 µg), chloramphenicol (30 µg), streptomycin (standard 10 µg, high-dose control 300 µg), and nystatin, used in antifungal tests (100 IU), are the most used disks. These standards were applied to ensure

consistency in test and positive control disks and to enhance the reliability of the results (CLSI, 2025; EUCAST, 2025).

3. Results and Discussion

Pediastrum boryanum (Turpin) Meneghini (Synonim: *Pseudopediastrum boryanum* (Turpin) E.Hegewald).

Pediastrum shows a colonial structure in freshwater. The colony may consist of 4-64 cells. The diameter of the colony varies according to the number of cells. If there are 16 or more cells, the cells are arranged in concentric rings. There are one or two horn-like extensions in the cells surrounding the colony. These structures are not seen in the central cells. There are pores between the cells in the colony. The cell walls are smooth, finely reticular or granular. The cells are multinucleated; scattered chloroplasts are single and parietal; one or more pyrenoids are present per cell. Asexual reproduction usually occurs after the production of biflagellate zoospores. Zoospores are found in vesicles in the cell and are released from the cell wall. The number of zoospores is species-specific and also depends on the physiological state of the cells. Sexual reproduction is rarely seen in *Pediastrum*. *Pediastrum* is usually planktonic. They are found in freshwater and pond environments. It has a wide distribution range from arctic to tropical climates. As the colonies grow, the colony sinks to the bottom. Their cell walls are unusual among green algae in having a significant silicon component. They also have a cell wall with a D-glucose and D-mannose crystalline component. Species are distinguished by cell size, shape, especially the status of peripheral cells, and colony morphology (Guiry and Guiry, 2024).

The latest taxonomic categorization of *P. boryanum* is as follows.

Empire: Eukaryota
Kingdom: Plantae
Subkingdom: Viridiplantae
Phylum: Chlorophyta
Subphylum: Chlorophytina
Class: Chlorophyceae
Order: Sphaeropleales
Family: Hydrodictyaceae
Genus: *Pediastrum*
Species: *P. boryanum* (Turpin) Meneghini (Synonim: *Pseudopediastrum boryanum* (Turpin) E.Hegewald) (Guiry and Guiry, 2024).

3.1 Antimicrobial Activity of Algal extraction solutions

Antibacterial Activity

Antibacterial activity tests have shown that the buffer extract of *P. boryanum* has highly effective antibacterial properties on *Listeria monocytogenes* ATCC 7644. It has been determined that *Listeria monocytogenes* ATCC 7644 strain is the most sensitive microorganism, while *Bacillus subtilis* ATCC 6633 strain is the most resistant microorganism; the most effective solvent is 0.5 M Tris-HCL pH:8.00 (Figure 1).

All the test microorganisms were affected by the extracts obtained from *P. boryanum* to varying degrees. It was recorded that this algae species was effective against the test microorganisms.

Antifungal Activity

Antifungal activity tests showed that all three extracts of *P. boryanum* had highly effective antifungal properties on *Candida albicans* ATCC 10239 (Figure 2).

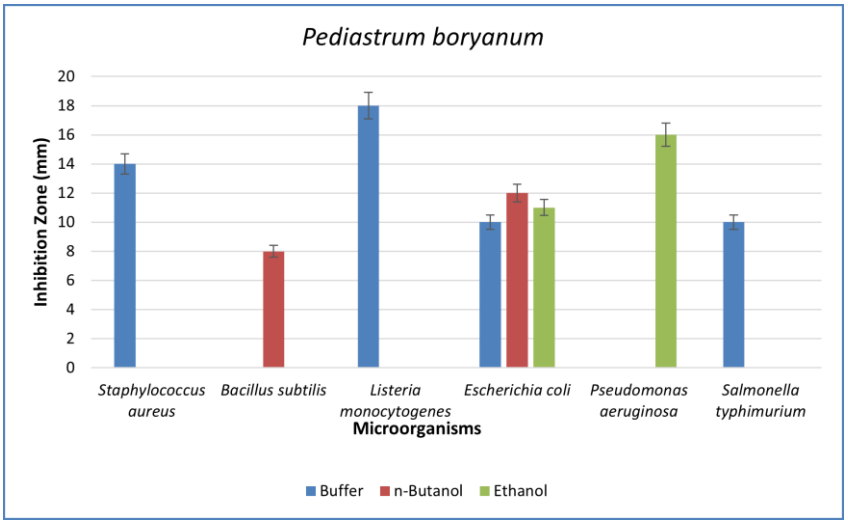

Figure 1. Inhibition zone diameter values created by *P. boryanum* extracts on test bacteria

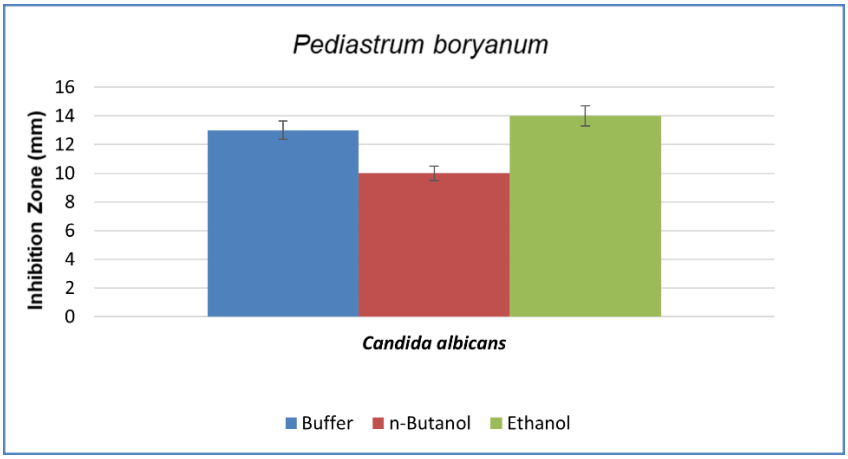

Figure 2. Inhibition zone diameter values created by *P. boryanum* extracts on the test fungu

3.2 Positive control

Penicillin, ampicillin, gentamicin, chloramphenicol, streptomycin and nystatin antibiotic disks were used as positive controls. In positive control studies, the inhibition zones formed by microorganisms against standard antibiotic disks were examined. As a result, it was observed that each microorganism formed an inhibition zone against the antibiotic disks to which it was affected or sensitive (Figure 3).

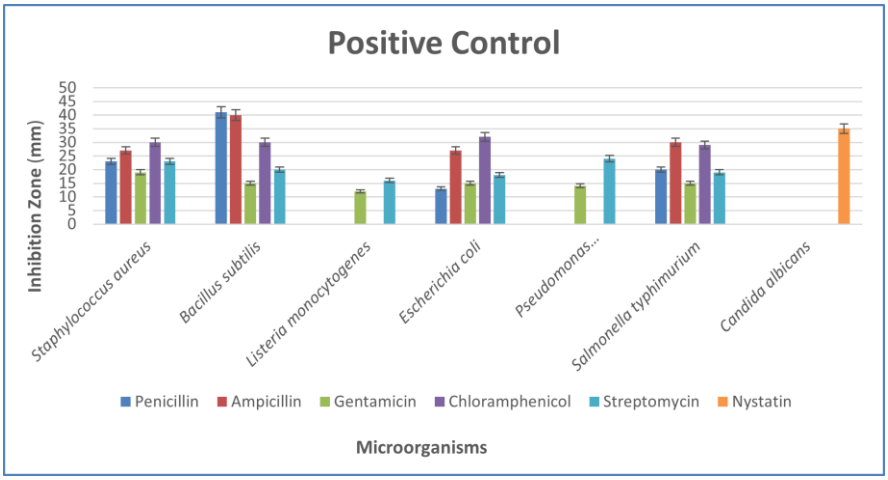

Figure 3. Antibacterial and Antifungal positive control test results

3.3 Negative control

Solvent impregnated disks and blank antibiotic disks were used as negative controls. No inhibition zones were observed in negative control studies.

In recent years, the increase in people's awareness about health, the inadequacy of synthetic drugs against newly emerging diseases and the detection of their side effects have increased the tendency towards the use of products obtained from natural substances (Yegin, 2017).

When the antimicrobial activity results were examined in our study; among the solvents used (0.5 M Tris-HCL pH: 8.00, N-butanol, Ethanol), the highest activity was in the extracts prepared with buffer solution and the lowest activity was in the extracts prepared with N-butanol. Among the test microorganisms used, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O 157:H7 and *Candida albicans* ATCC 10239 were susceptible, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* CCM 5445 were intermediately susceptible, and *Bacillus subtilis* ATCC 6633 was resistant.

In the study where the antioxidant capacity of phenolic compounds found in *P. boryanum* grown in six different culture media, primarily standard BG11, was examined, DPPH and ABTS methods were used and total phenolic compounds were also examined. The strongest scavenging activity was determined in the DPPH method, and it was recorded that it inhibited peroxidase well in the ABTS method. In the study, it was stated that *P. boryanum* could potentially be an important source especially in terms of gallic, protocatechuic, chlorogenic, and hydroxybenzoic (Corrêa da Silva et al., 2020). *P. boryanum* used in our study was also grown in BG11 medium and subjected to antimicrobial tests, and it was found to be effective on the tested microorganisms (Figure 1-3). In this respect, it is seen that the phenolic compounds present in *P. boryanum* influence the good biological activity recorded in the tests.

Scientists have conducted antimicrobial studies with various algae species and have achieved important results.

In the study conducted by Vehapi et al. in 2021 with *Ulva lactuca*, they investigated the antifungal effects of algal extracts against *Fusarium oxysporum* and the antibacterial effects against bacterial microorganisms *Proteus mirabilis* BC6624, *Mycobacterium smegmatis* RUT and *Aeromonas hydrophila* ATCC7965. In the study, the agar disk diffusion method was used and the inhibition zone diameters against *Fusarium oxysporum* were measured as 47.00-46.50 mm in 20 and 40 µL. It was reported that *P. mirabilis* and *M. smegmatis* were resistant to DMSO extracts of *Ulva lactuca,* while *A. hydrofila* was susceptible (Vehapi et al., 2021). In our study, it was determined that *Listeria monocytogenes* ATCC 7644 strain was the most sensitive microorganism, *Bacillus subtilis* ATCC 6633 strain was the most resistant microorganism, and the most effective solvent was 0.5 M Tris-HCL pH: 8.00.

In the study conducted by Saleh and Al Mariri 2017, the antimicrobial activities of *Halimeda* sp. were evaluated. The methanol extract of algae used in the study was examined for its antimicrobial activities against Gram-positive bacteria (*B. subtilis*, *S. aureus* and *B. cereus*), Gram-negative bacteria (*P. aeruginosa*, *E. coli*), yeast (*C. albicans*) and fungi (*A. niger*). As a result, they observed that *Halimeda* sp. inhibited the growth of *B. subtilis*, *S. aureus* and *B. cereus* (Saleh and Al Mariri, 2017).

In the study by Al-Wakeel et al. (2024), selenium nanoparticles (SeNPs) were synthesized using *Pediastrum boryanum* algal extract, and their antimicrobial activities were evaluated by the disk diffusion method. Ten grams of dried algae were extracted in 100 mL of solvent for 24 hours, then mixed with 50 mL of 1 mM sodium selenite solution and incubated at 37 °C for 12 hours. Standard antibiotic disks, including penicillin G, ampicillin, gentamicin, chloramphenicol, streptomycin, and nystatin (Oxoid, UK), were used as positive controls. The tested microorganisms included Gram-positive bacteria (*B. subtilis, S. aureus, B. cereus*), Gram-negative bacteria (*P. aeruginosa, E. coli*), yeast (*C. albicans*), and fungi (*A. niger*), with 20–50 µL of extract applied per disk. Bacteria were incubated at 35–37 °C for 18–24 hours, and *C. albicans* at 30 °C for 24–48 hours. The results showed that Gram-positive bacteria were more sensitive to both the extract and SeNPs, while Gram-negative bacteria were relatively more resistant (Al-Wakeel et al., 2024).

Antimicrobial activities of extracts of some algae belonging to Rhodophyta (*Gracilaria verrucosa*, *Gelidiella acerosa* and *Hypnea musciformis*) against gram-negative (*Shigella flexneri* and *Salmonella typhi*) and gram-positive (*Enterococcus aerogenes*, *Salmonella paratyphi*, and *Staphylococcus epidermidis*) bacteria were determined by disk diffusion method. Ethanol, methanol, water and chloroform were used as solvents for the extraction of algae, and it was recorded that none of the water extracts of *G. verrucosa* showed activity. On the other hand, it was reported that chloroform extracts formed the highest inhibition zone (21 mm) against *S. paratyphi* (Varier et al., 2013). In our study, the most effective solvent was observed to be 0.5 M Tris-HCL pH:8.00.

In the study investigating the antibacterial effects of methanol, ethanol and acetone extracts of *Cladophora glomerata* on *Bacillus subtilis* and *Streptococcus mutans*,

it was reported that all extracts showed antibacterial effects, but ethanol and methanol (1.85 cm zone diameter) were more effective than acetone (0.7 cm zone diameter) and both solvents (ethanol and methanol) could be used as good solvents in the antibacterial activity test (Sadıq et al., 2016).

In the study where the antimicrobial effects of *Enteromorpha intestinalis* were investigated on five different gram negative and positive bacteria (*Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus subtilis*, *Proteus mirabilis* and *Pseudomonas aeruginosa*) by disk diffusion method, it was stated that *P. aeruginosa* from gram negative bacteria was more resistant than *P. mirabilis* and *S. typhimurium*, *B. subtilis* from gram positive bacteria was more sensitive than *S. aureus* and it was stated that *E. intestinalis* has the possibility of being used as a new natural antimicrobial and antihemolytic agent for pharmaceutical industries (Soltani et al., 2012). In our study, it was observed that *P. boryanum* extracts were effective on both bacteria and fungi and it was determined that it can be used as a natural agent in this respect.

4. Conclusion

Recently, the indiscriminate use of drugs used in the treatment of infectious diseases has led to pathogens developing resistance to antimicrobial agents, thus increasing the rate of disease in humans. In this case, it has become necessary to discover new antimicrobial substances from different sources and investigate their structures.

P. boryanum used in our study appears to be a useful organism in terms of its antibacterial and antifungal properties, and it is thought that it holds potential for pharmaceutical development through mass cultivation and isolation of its bioactive metabolites.

Author Contribution

Demiriz Yücer, T.: Idea and concept, Literature review, Laboratory analyses, Comments, Writing the article.

Pabuçcu, K.: Idea and concept, Laboratory analyses, Review and editing of the article.

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Conflict of Interest Statement

All the authors declare no conflict of interest.

Ethics Standards

No Ethics Committee Decision is required for this study.

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