



This article is cited as: Akkoyunlu A., Dülger G., Dülger B. (2025). Antibacterial Activity of *Laetiporus sulphureus* Extracts Against Multidrug Resistant Bacterial Isolates. *Mantar Dergisi*, 16(1)1-7

Geliş(Received) :30.10.2024
Kabul(Accepted) :19.11.2024

Research Article
Doi: 10.30708/mantar.1575046

Antibacterial Activity of *Laetiporus sulphureus* Extracts Against Multidrug Resistant Bacterial Isolates

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Abstract: Ethanol extracts from *Laetiporus sulphureus* (Bull.) Murrill (*Polyporaceae*) were tested for their antibacterial activity against multidrug-resistant bacterial isolates using agar well diffusion and dilution procedures. According to the findings, *L. sulphureus*'s antibacterial activity differed depending on the extract concentration. While no activity was observed at other dosages, the ethanolic extract demonstrated inhibitory zones ranging from 10.2 to 17.4 mm at 200 mg/mL; 8.2 to 12.6 mm at 100 mg/mL; and 9.2 to 9.8 mm at 50 mg/mL. *Klebsiella pneumoniae* (10.2 mm at 200 mg/mL) was the least susceptible to the extract, while *Bacillus subtilis* (17.4 mm at 200 mg/mL) was the most sensitive. The extract's minimum inhibitory concentration (MIC) against *Staphylococcus aureus*, *B. subtilis*, and *K. pneumoniae* ranged between 25 mg/mL and 200 mg/mL. For *B. subtilis*, *S. aureus*, and *Pseudomonas aeruginosa*, the minimum bactericidal concentration (MBC) of the extract was recorded at 50 mg/mL and 200 mg/mL, respectively; however, no MBC was found for *K. pneumoniae*. *S. aureus* exhibited the highest level of resistance (70%), while *P. aeruginosa* demonstrated the lowest level of resistance (10%). Pefloxacin, ciprofloxacin, septrin, and sparfloxacin were identified as the most potent antibiotics. The findings suggest that *L. sulphureus* has potential as an antibacterial agent, though its effectiveness varies according to extract concentration and bacterial species.

Keywords: *Laetiporus sulphureus*, Antibacterial activity, Multidrug-resistant bacterial isolates

Çoklu İlaça Dirençli Bakteri İzolatlarına Karşı *Laetiporus sulphureus* Ekstraktlarının Antibakteriyel Aktivitesi

Öz: *Laetiporus sulphureus* (Bull.) Murrill (*Polyporaceae*) etanol ekstraktlarının, çoklu ilaca dirençli bakteri izolatlarına karşı antibakteriyel aktivitesi agar kuyucuk difüzyon ve dilüsyon yöntemleri kullanılarak test edilmiştir. Bulgulara göre, *L. sulphureus*'un antibakteriyel aktivitesi ekstrakt konsantrasyonuna bağlı olarak değişiklik göstermektedir. Diğer dozlarda aktivite gözlenmezken, etanolik ekstrakt 200 mg/mL'de 10,2 ile 17,4 mm arasında, 100 mg/mL'de 8,2 ile 12,6 mm arasında ve 50 mg/mL'de 9,2 ile 9,8 mm arasında inhibisyon zonları göstermiştir. Ekstrakta en az duyarlı bakteri 200 mg/mL'de 10,2 mm ile *Klebsiella pneumoniae* iken, en duyarlı bakteri 200 mg/mL'de 17,4 mm ile *Bacillus subtilis* olmuştur. Ekstraktın *Staphylococcus aureus*, *B. subtilis* ve *K. pneumoniae*'ye karşı minimum inhibitör konsantrasyonu (MİK) 25 mg/mL ile 200 mg/mL arasında değişmektedir. *B. subtilis*, *S. aureus* ve *Pseudomonas aeruginosa* için ekstraktın minimum bakterisidal konsantrasyonu (MBK) sırasıyla 50 mg/mL ve 200 mg/mL olarak

kaydedilmiştir; ancak *K. pneumoniae* için herhangi bir MBK bulunamamıştır. *S. aureus* en yüksek direnç seviyesini (%70) gösterirken, *P. aeruginosa* en düşük direnç seviyesini (%10) göstermiştir. Pefloksasin, siprofloksasin, septrin ve sparfloksasin en güçlü antibiyotikler olarak tanımlanmıştır. Bulgular, *L. sulphureus*'un bir antibakteriyel ajan olarak potansiyel taşıdığını, ancak etkinliğinin ekstrakt konsantrasyonuna ve bakteri türüne göre değiştiğini göstermektedir.

Anahtar kelimeler: *Laetiporus sulphureus*, Antibakteriyel aktivite, Çoklu ilaca dirençli bakteri izolatları

Introduction

According to recent statistics, a significant number of people are using alternative medicine in various ways. One of the most accessible forms of complementary and alternative medicine is macrofungi. Both wild and farmed mushrooms contain a wide range of biomolecules with nutritional and/or medicinal benefits (Brochers et al., 2004; Lindequist et al., 2005; Poucheret et al., 2006; Kalac, 2009). Mycelia, spores, and fruiting bodies collect a variety of bioactive chemicals that have antiviral, antibacterial, antidiabetic, antioxidant, anticancer, liver-protecting, antifibrotic, and anti-inflammatory properties (Alves et al., 2012).

The edible wood-rotting *basidiomycete* fungus *Laetiporus sulphureus* (Bull.) Murrill (*Polyporaceae*) is also known as sulphur shelf, sulphur polypore, or "chicken of the woods". It is also called "kükürtmantarı" in Turkish. Although it is found worldwide, it is predominantly seen in the tropical and subtropical regions of Europe, Asia, and North America (Petrovic et al. 2013,; Sesli et al., 2020). The fungus has a distinct texture and scent; characteristics that have influenced its long-standing use in Oriental culture as a valuable food source (Khatua et al., 2017). Furthermore, because of this fungus's medicinal qualities, it is also valued as a folk medicine, particularly in Asia and Europe (Zjawiony, 2004). In Europe, traditionally, fruit bodies are widely used to treat rheumatism, coughs, stomach cancer, and pyretic illnesses (Grienke et al., 2014). During normal field trips, it was discovered that *L. sulphureus* is used to treat coughs (prepared as a tea) and wounds (in the form of pomade). Consequently, the aim of this study was to ascertain the antibacterial effect of ethanolic extracts of *L. sulphureus* gathered from Türkiye, which locals use to treat various ailments.

Material and Metod Fungal Materials

In July and August of 2019, the macrofungus *Laetiporus sulphureus* was obtained from Sinekli Yaylası, Duzce, Türkiye. The author's personal collection of voucher specimens (GD-122-3) of the macrofungus has been stored in the Department of Medical Biology at Duzce University.

Preparation of Crude Extracts

Macrofungus samples were oven-dried at 40 °C and after that pulverised. Soxhlet apparatus was used to extract each dry powdered macrofungal material (50 g) using 150 mL of 95% ethanol (Merck, Darmstadt, Germany) over the course of 24 hours. Filter paper No. 1 from Whatman was employed to strain the extract, and a rotary evaporator operating under vacuum at 55 °C was used to evaporate the filtrate solvent (yield 37.4% for ethanol). The dry extract that resulted was kept at -20 °C in labelled, sterile screw-capped bottles. To get a 200 mg/mL concentration, 1 g of the extract was dissolved in 5 mL of DMSO (dimethyl sulfoxide). As stated by Ahmad and Beg (2001), additional doses of 100, 50, 25, 12.5, and 6.25 mg/mL were created using the dilution approach.

Microorganisms

In this study, *Bacillus subtilis*, *Staphylococcus aureus* as Gram-positive bacteria and *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* as Gram-negative bacteria were used. The bacterial cultures were acquired from the Faculty of Medicine, Medical Biology Research Laboratory, Düzce University, Düzce, Türkiye.

Antibiotic Susceptibility Testing of the Test Microorganisms

In compliance with the National Committee Laboratory Standards (2000) recommendation, antimicrobial disc tests were conducted on the isolates utilising the subsequent antibiotic discs (ampiclox (30 µg), amoxicillin (30 µg), zinnacef (20 µg), rocephin (25 µg), ciprofloxacin (10 µg), augmentin (20 µg), sparfloxacilin (30 µg), erythromycin (10 µg), streptomycin (30 µg), gentamycin (10 µg), septrin (30 µg), chloramphenicol (25 µg), pefloxacin (10 µg), tetracycline (30 µg) and ofloxacin (30 µg)). Based on the diameter of the inhibitory zones surrounding the antibiotic discs.

Antibacterial Susceptibility Testing of the Extracts with the Test Organisms

The test microorganisms were inoculated in Mueller Hinton Broth (Oxoid) to prepare the inocula, and then incubated at 37 °C for 24 hours. After the incubation, the cultures were diluted to the McFarland turbidity standard of [0.5]. Using a glass rod spreading technique, 0.2 mL of the colonies were further diluted in normal

saline and seeded onto solidified Mueller Hinton Agar (Oxoid). The agar well technique was used to assess each extract's capacity to stop the growth of the clinical test bacteria. Mueller Hinton Agar plates that had been infected were left to dry. Next, using a 4 mm cork borer, wells were drilled into the surface of inoculated agar plates. Using a Pasteur pipette, 0.2 mL of each extract's various concentrations were added to the well. The ensuing zones of inhibition were not allowed to overlap since the wells were separated enough apart. The zones of inhibition that resulted from the triplicate execution of the experiment were noted. Penicillin, Ampicillin, and Streptomycin served as positive controls.

Determination of Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC)

For every test microorganism, MIC of the ethanol extract was ascertained in triplicate at different concentrations ranging from 200 to 3.125 mg/mL. A loopful of the test microorganisms that had been previously diluted to the [0.5] McFarland turbidity standard was added to the tubes along with 1 mL of Mueller Hinton Broth (Oxoid). The test microorganisms were planted into a tube containing Mueller Hinton Broth only as a control. Following 24-hour incubation at 37 °C, turbidity was measured in each tube to check for growth.

The MIC of the macrofungal extract on the clinical bacterial isolates was performed according to Ajaiyeoba et al. (2003). To put it briefly, 1 mL of bacterial culture was transferred from the mixture used to assess the MIC tubes. These did not exhibit any growth; therefore, they were subcultured onto Mueller Hinton Agar (Oxoid) and incubated for 24 hours at 37 °C. The concentration at which no single bacterial colony was found after incubation was designated as MBC. Ampicillin and streptomycin were prepared at concentrations ranging from 128 to 0.25 µg/mL to be used as standard antibacterial agents.

Data Analyses

In this study, a one-way ANOVA (Analysis of Variance) was conducted to evaluate if there were significant differences in the inhibition zones at different concentrations of the plant extract against various bacterial strains. The ANOVA results showed a statistically significant difference between the groups ($F = 26.515$, $p < 0.01$). To further investigate these differences, a post-hoc Tukey HSD test was conducted. The Tukey test revealed that the inhibition zones at higher concentrations (e.g., 200 mg/mL) were significantly different from those at lower concentrations (e.g., 25 mg/mL and below) with p -values < 0.001 .

A Pearson correlation analysis was performed to examine the relationship between MIC and MBC values. The analysis revealed a perfect positive correlation between MIC and MBC ($r = 1.000$, $p < 0.01$). This result indicates that an increase in MIC values leads to a proportional increase in MBC values, and the relationship is statistically significant.

IBM SPSS Statistics 27 software was used to perform the analyses.

Results

The ethanol extract of *L. sulphureus* was used in this investigation and examined using the agar well diffusion and dilution method. When tested against various bacteria, the extracts demonstrated potential antibacterial properties. It was observed that the antibacterial activity of *L. sulphureus* depended on both extract concentration and bacterial species.

According to Table 1, inhibition zones of the extract varied from 8.2 mm at 100 mg/mL against *K. pneumoniae* to 17.4 mm at 200 mg/mL against *B. subtilis*. *K. pneumoniae* was the least susceptible bacterial culture to the extract, whilst the most sensitive bacterium was *B. subtilis*. At 200 mg/mL, the extract exhibited a moderate efficacy against *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. The efficacy of the extract at 200 mg/mL showed stronger inhibition against *S. aureus*, *E. coli* and *B. subtilis* isolates compared to standard antibiotics.

Table 2 compares the MIC and MBC values of the extract and antibiotics (streptomycin and ampicillin). The MIC value of the ethanolic extract was found to range from 25 mg/mL for *S. aureus* and *B. subtilis* to 200 mg/mL for *K. pneumoniae*. While the MBC of the ethanolic extract was 50 mg/mL for *S. aureus* and *B. subtilis* and 200 mg/mL for *P. aeruginosa*, no MBC was observed for *K. pneumoniae*. The higher MIC and MBC values of the extract compared to standard antibiotics suggest that it is less effective.

After being tested for antibiotic sensitivity, the bacterial strains employed in this investigation were found to have varied degrees of antibiotic resistance, as shown in Table 3. The microbes exhibited selected resistance to ten distinct antibiotics that were utilised. *S. aureus* was the most resistant bacterium strain (70%), whilst *P. aeruginosa* (10%) was the least resistant. Septrin, pefloxacin, ciprofloxacin, and sparfloxacin were the most effective antibiotics.

Table 1. Antibacterial activity of *L. sulphureus* ethanolic extract (zones of inhibition are measured in millimeters)
Inhibition zones (mm)*

Test microorganisms	Plant extract						Standard antibiotics		
	Concentrations (mg/mL)						P	AM	ST
	200	100	50	25	12.5	6.25			
<i>Escherichia coli</i>	14.6 ^a	12.4 ^a	9.8 ^b	0	0	0	7.0	11.0	12.0
<i>Staphylococcus aureus</i>	16.6 ^a	11.8 ^a	9.0 ^b	0	0	0	12.0	14.0	13.0
<i>Pseudomonas aeruginosa</i>	11.4 ^a	7.4 ^b	0	0	0	0	8.0	9.0	11.0
<i>Bacillus subtilis</i>	17.4 ^a	12.6 ^a	9.2 ^b	0	0	0	10.0	12.0	16.0
<i>Klebsiella pneumoniae</i>	10.2 ^b	8.2 ^b	0	0	0	0	9.0	13.0	17.0

*Includes diameter of well (4 mm). P: Penicillin G (10 µg/mL); AM: Ampicillin (20 µg/mL); ST: Streptomycin (10 µg/mL); ^a p<0.01; ^b p<0.05

Table 2. MIC and MBC of ethanolic extract of *L. sulphureus*

Test microorganisms	Extract (mg/mL)	MIC (MBC)	
		Standards (µg/mL)	
		Streptomycin	Ampicillin
<i>Escherichia coli</i>	50 (100)	4.0 (4.0)	8.0 (16.0)
<i>Staphylococcus aureus</i>	25 (50)	2.0 (4.0)	1.0 (2.0)
<i>Pseudomonas aeruginosa</i>	100 (200)	1.0 (1.0)	16.0 (32.0)
<i>Bacillus subtilis</i>	25 (50)	4.0 (8.0)	8.0 (16.0)
<i>Klebsiella pneumoniae</i>	200(-)	4.0 (8.0)	16.0 (32.0)

Table 3. Antibiotic Susceptibility Test

Test microorganisms	Antibiotics										Resistance (%)	
	E	CN	CPX	SXT	PEF	ST	RO	Z	AM	APX		
Gram (+) Bacteria												
<i>Staphylococcus aureus</i>	R	R	R	R	S	S	R	S	R	R		70
<i>Bacillus subtilis</i>	S	S	S	S	S	S	S	R	R	S		20
Gram (-) Bacteria	AM	STX	OFX	CN	PEF	SP	CH	T	AU	CPX		
<i>Escherichia coli</i>	S	S	R	R	S	S	S	R	R	S		40
<i>Pseudomonas aeruginosa</i>	S	S	S	S	S	S	S	R	S	S		10
<i>Klebsiella pneumoniae</i>	R	S	S	S	S	S	R	S	S	S		20

APX: Ampiclox; AM: Amoxicillin; AU: Augmentin; CH: Chloramphenicol; CN: Gentamisin; CPX: Ciprofloxacin; E: Erythromycin; OFX: Ofloxacin; PEF: Pefloxacin; RO: Rocephin; SP: Sparfloxacin; ST: Streptomycin; STX: Septrin; T: Tetracycline; Z: Zinnacef.

S: Susceptible; R: Resistant

Discussions

In this study, the antibacterial effectiveness ethanolic extracts derived from the macrofungus *Laetiporus sulphureus* (Bull.) Murrill (*Polyporaceae*) was evaluated against multidrug-resistant bacterial isolates using agar well diffusion and dilution methods. The findings suggest that *L. sulphureus* has the potential to be noted as an antibacterial agent, though its efficacy varies based on the type of bacterial species and the concentration of the extract. The inhibition zones in the

Table 1 decrease as the concentration of the extract decreases, demonstrating a clear dose-dependent effect. This trend is consistent with the higher MIC and MBC values for the plant extract in the Table 2, reinforcing those higher concentrations of the extract are necessary to achieve comparable effects to antibiotics.

In an earlier investigation, six Gram-negative bacteria, seven Gram-negative bacteria, and one yeast culture were used to test the *L. sulphureus* ethanolic extract's antibacterial efficacy (Turkoglu et al., 2002).

Gram-positive bacteria including *Bacillus cereus*, *Micrococcus luteus*, *B. subtilis* and *M. flavus* were strongly inhibited from growing by the macrofungus, which also showed a restricted antibacterial spectrum against Gram-negative bacteria. The inhibition zones varied between 10 and 23 mm, with *M. flavus* exhibiting the highest sensitivity, demonstrating a zone of 23 ± 1 mm. In a separate study, extracts of *L. sulphureus* in hexane and chloroform displayed antifungal and antibacterial properties, with the hexane extract proving to be more effective (Sinanoglu et al., 2015). Alves et al. (2012) noted that although extracts were generally less effective against Gram-negative bacteria, they did manage to inhibit the growth of *E. coli*. Additionally, Petrovic et al. (2013) discovered that extracts of *L. sulphureus* (using methanol, acetone, and dichloromethane) exhibited strong antimicrobial activity against eight bacterial strains, eight fungal strains, and *Aspergillus flavus in situ* in tomato paste.

In an additional study, *L. sulphureus* extracts exhibited antimicrobial activity against *Geotrichum candidum*, *Candida albicans*, *Aspergillus niger*, *A. fumigatus*, *Fusarium oxysporum*, and *Curvularia clavata*, as well as Gram-positive bacteria like *Streptococcus pyogenes* and *S. aureus*, and Gram-negative bacteria including *K. pneumoniae*, *E. coli*, *Shigella enterica*, and *P. aeruginosa*. Water and ethanol extracts showed stronger antibacterial activity with lower MIC values (MIC = 15.6-62.5 $\mu\text{g/mL}$ for ethanol and MIC = 15.6-125 $\mu\text{g/mL}$ for water) (Younis et al., 2019). Parvus et al. (2011) also reported that *L. sulphureus* water-ethanol extract had antifungal activity against *Fusarium oxysporum* f. sp. *tulipae*, *Aspergillus niger*, *Penicillium gladioli*, *Sclerotinia sclerotiorum* and *Botrytis cinerea* and the MIC values were comparable to fluconazole, a well-known antifungal. The results of the present study are in agreement with the results of the previously mentioned literature. Although previous studies have demonstrated the antibacterial properties of *L. sulphureus* against various bacterial strains, the present study offers new perspectives by specifically investigating the effects of *L. sulphureus* extracts on multidrug-resistant hospital isolates. Apart from the slight differences in the effect of *L. sulphureus* ethanolic extract, it is possible that the peptidoglycan layer of Gram-negative bacteria is covered by a

lipopolysaccharide (LPS) layer, which prevents the spread of the extract to the bacteria. According to many studies, the LPS layer is crucial for selective permeability (Li et al., 2010). In addition, detoxifying and hydrolytic enzymes in the periplasmic space of Gram-negative bacteria can absorb inert foreign substances from their environment. Furthermore, Gram-positive bacteria are predicted to be more resistant to these extracts than Gram-negative bacteria due to their thicker, hydrophilic, porous structure and lack of outer lipopolysaccharide membrane (Ren et al., 2014; Duvnyak et al. 2016). In another study, it was found that *L. sulphureus* is rich in various minerals, vitamins (B, D, and E), fibers, and a range of amino acids, along with significant carbohydrate content, including trehalose, mannitol, and fructose (Khatua et al., 2017). Additionally, it contains polyunsaturated fatty acids such as oleic acid, palmitic acid, and linoleic acid.

L. sulphureus is rich in triterpenes, phenolics, polysaccharides and unsaturated fatty acids, which contribute to its diverse biological effects, including antimicrobial, antitumor, antioxidant, immunomodulatory and anti-inflammatory properties. (Fan et al., 2014; Wang et al., 2015; Sinanoglu et al., 2015; Acharya et al., 2016).

The findings of this study show promising antibacterial activity due to the bioactive components that may be responsible for the activity of ethanolic extract of *L. sulphureus* against multidrug-resistant bacteria. However, further research is required to purify specific bioactive components and then convert them into chemotherapeutic agents.

Author contributions

The contributions of all authors are considered equal in the preparation of the research presented in the manuscript.

Conflicts of interest

The authors declare no competing interests.

Ethical Statement

It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Ayşegül AKKOYUNLU, Görkem DÜLGER, Başaran DÜLGER)

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