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Antibacterial Effect of *Liquidambar Orientalis* Miller Resin on Nosocomial Infection Agents

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Abstract

Aim: Today, nosocomial infections are becoming an important problem due to antibiotic resistance, and high mortality rates. There is a need for novel and natural antimicrobial agents that can be used in the treatment of nosocomial infections. In this study, it was aimed to investigate the antibacterial effect of *Liquidambar Orientalis* Miller (Sweetgum tree) resin, an endemic species grown only in our country, on nosocomial infection-caused bacteria.
Material and Methods: In our study, *Liquidambar Orientalis* Miller resin was obtained from the Marmaris district of Muğla province. The antibacterial effect of this resin on *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 700603, *S. haemolyticus* ATCC 43252, *A. baumannii* ATCC 19606 and *E. faecalis* ATCC 29212 bacteria, was investigated by agar well diffusion method. Penicillin (10U), Streptomycin (10µg), and Vancomycin (30µg) antibiotics were used as a control in the study.
Results: As a result of the study, it was observed that the sweetgum tree resin exhibited an inhibition zone (average: 23.2 mm) on all tested bacteria. It has been determined that sweetgum tree resin formed a higher inhibition zone on Gram-positive bacteria than on Gram-negative bacteria.
Conclusion: In studies with different drugs (balsam, leaf extracts, etc.) of the *Liquidambar Orientalis* tree, it has been reported that they have antibacterial effects on various bacteria. However, in this study, it was determined that *Liquidambar Orientalis* Miller resin has an antibacterial effect on nosocomial infection agent bacteria. Our results show that the resin of *Liquidambar Orientalis* has the potential to be used as an alternative to antibiotics for nosocomial infections.

Keywords: Nosocomial infectious agent, Opportunistic pathogen, Antibacterial effect, Agar well diffusion method, Sweetgum tree, *Liquidambar orientalis*

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Introduction

Nosocomial infections are infections that are not yet in the incubation period when a patient is hospitalized, develop later, and sometimes occur after the patient is discharged (1). Nosocomial infections cause serious problems such as an increase in the period of hospitalization and mortality rate, an increase in the cost of the treatment process, and cause multi-antibiotic resistance in microorganisms (2,3). With the increasing hospitalizations in the current COVID-19 pandemic, there has been an increase in deaths due to secondary infections caused by nosocomial agents, especially in intensive care patients (4,5). Multi-drug resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus* species, and carbapenem-resistant *Klebsiella pneumonia* are important microorganisms that cause nosocomial infections, and treatment success rates are gradually decreasing due to the antibiotic resistance they have developed (6,3). Consequently, there is a need for new, natural, and economical antimicrobial agents that can be used in the treatment of nosocomial infections.

Liquidambar Orientalis Miller (Sweetgum tree) is an endemic species native to Turkey and is the only member of the *Hamamelidaceae* family in our country (7). It is known in the literature that different drugs of sweetgum tree are prepared and used in the treatment of various diseases. For example, it has been reported that sweetgum balsam is used in the treatment of wounds, disinfection with steam in upper respiratory tract diseases such as asthma and bronchitis, powder and lozenges as an expectorant, pomade and patch in skin diseases such as fungus and scabies, and as a pain reliever in ulcer disease (8-11). In studies on the chemical structure of sweetgum trees, it has been determined that sweetgum oil contains high molecular compounds such as acid, alcohol, ester, and phenol. Studies using various extracts of the leaves of the tree or balsam of the tree have been shown to have antibacterial effects against various Gram-positive and Gram-negative bacteria (12- 15).

This study, it was aimed to investigate the antibacterial effect of *Liquidambar Orientalis* resin, which is an endemic species grown only in our country, on nosocomial infection-caused bacteria.

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Material And Methods

Plant Species and Bacteria Used in the Study

In our study, resin belonging to the *Liquidambar Orientalis* Miller species was obtained from the Marmaris district of Muğla province in November 2018. In the study, *E. faecalis* ATCC 29212, *S. haemolyticus* ATCC 43252, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 700603, and *A. baumannii* ATCC 19606 were used as nosocomial infection agent bacteria.

Antibacterial Activity Test

Agar-well diffusion method was used to determine the antibacterial effect of sweetgum tree resin on nosocomial infection agents. 24-hour fresh cultures of all bacteria to be used in the test were prepared in Mueller Hinton Broth (MHB), and the bacterial concentration was adjusted as 0.5 McFarland (1.5x 10^8 CFU/ml) (16). Mueller Hinton Agar (MHA) media were prepared and wells of 6 mm diameter were drilled into these media with sterile glass Pasteur pipettes. 100 µl of the bacterial cultures were transferred to the medium and inoculated with the spread plate method. 50 µl of sweetgum resin was added to the drilled wells. In the tests, broad-spectrum penicillin (10U) antibiotic for Gram-positive and Gram-negative bacteria, narrow-spectrum streptomycin (10µg) antibiotic for Gram-negative bacilli, and narrow-spectrum vancomycin (30 µg) antibiotic for Gram-positive cocci were used as positive controls. All Petri dishes were then incubated at 37 °C for 24 hours. Inhibition zone diameters (mm) were determined by measuring the inhibition zones formed at the end of the incubation with a caliper.

Results

As a result of the study, it was observed that the sweetgum tree resin exhibited a zone of inhibition (average: 23.2 mm) on all tested bacterial strains. Compared to the control groups, the highest inhibition zone (31 mm) was on *A. baumannii* ATCC 19606, and the lowest inhibition zone (12 mm) was on *P. aeruginosa* ATCC 27853. The inhibition zone diameters of

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sweetgum tree resin on all bacteria and the inhibition zone diameters of antibiotics on all bacteria are shown in Figure 1 (Figure 1).

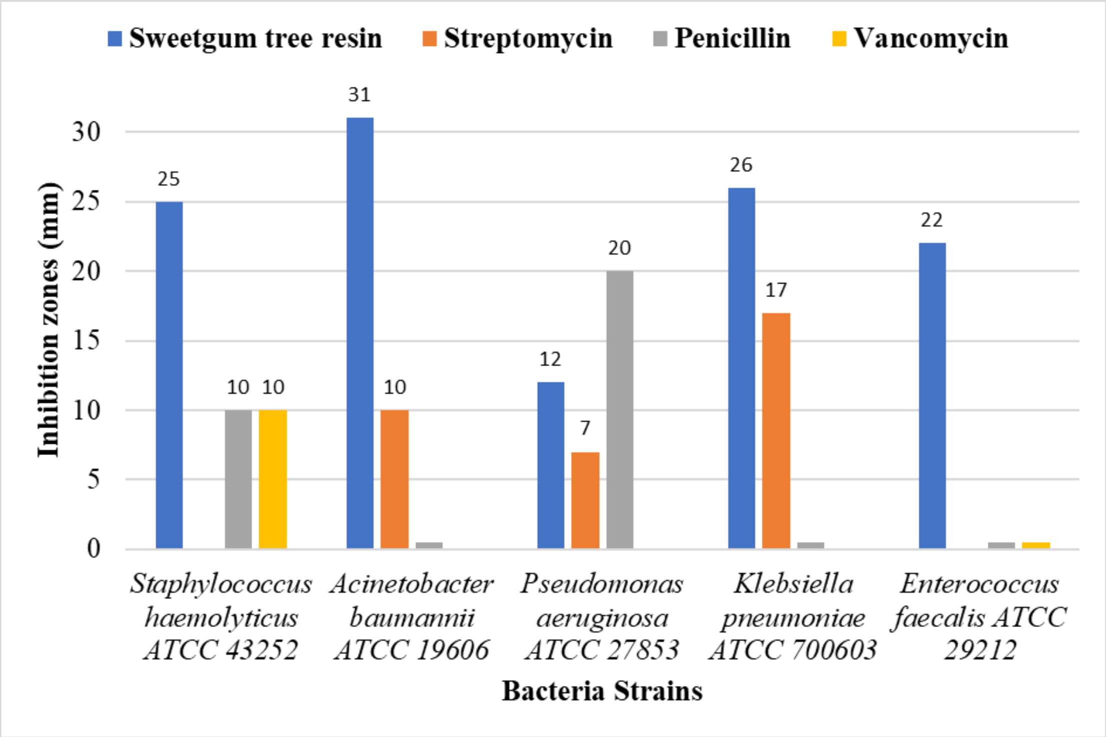

Figure 1: Inhibition zone diameters (mm) of bacteria against sweetgum tree resin and antibiotics (Streptomycin, Penicillin, Vancomycin).

When the inhibition effects of sweetgum tree resin on Gram-positive and Gram-negative bacteria were compared, it was determined that it exhibited a higher inhibition zone on Gram-positive bacteria (average 23.5 mm) than Gram-negative bacteria (average 19.6 mm). In addition, according to the antibacterial activity test results, *S. haemolyticus* ATCC 43252 and *E. faecalis* ATCC 29212 strains of bacteria used in the tests were found to be resistant to vancomycin and penicillin. It was observed that sweetgum tree resin exhibited a high inhibition zone (25 mm and 22 mm) on these antibiotic-resistant strains.

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Discussion

Liquidambar Orienta Miller is an endemic species of the *Hamamelidaceae* family, *Bucklandioidae* subfamily, and *Liquidambar* genus, which is distributed in Turkey. It has been reported that this species has good antiseptic and parasitic properties and is used in the respiratory tract and lung diseases such as asthma and bronchitis, and skin disorders such as scabies and fungi in the form of pomade and patch. Some studies determined the anti-ulcerogenic, antioxidant, antimicrobial, and antithermitic activities of the balsam, leaves, or essential oil of this species (9, 17, 18). In the literature, there are research studies on the antimicrobial effect of different drugs (sweetgum balsam or leaf extracts) of *Liquidambar orientalis* (11,12,14,15,19-22).

In one of the most comprehensive studies on the antibacterial activity of sweetgum balsam; 10% concentration of the balsam has an antibacterial effect against *Bacillus brevis, Bacillus cereus, Bacillus subtilis, Corynebacterium xerosis, Enterobacter aerogenes, Staphylococcus aureus* bacteria, 1% concentration has antibacterial effect against *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Proteus* udderogenes fluse specified (19). In another study, ethanol extracts of sweetgum leaves were prepared, and it was determined that this extract had a strong antibacterial effect, especially on Methicillin-resistant *Staphylococcus aureus* (12).

Aşkun et al. investigated the antimycobacterial activity and balm content of *Liquidambar Orientalis* balsam and determined that the balsam had a high antibacterial effect against four of the six *Mycobacterium tuberculosis* strains tested (14). Keskin and Güvensen investigated the antibacterial effects of different extracts of *Liquidambar orientalis* left by the disc diffusion method. As a result of the study, they stated that *K. pneumoniae* showed the best inhibition zone (32mm) against ethanolic extracts of *Liquidambar Orientalis*. In addition, it has been observed that the extract has an antibacterial effect on *P. aeruginosa*, (24 mm), *S. typhimurium* (24 mm), *S. epidermidis* (24 mm), *E. faecalis* (21 mm), and *S. aureus* (19 mm) bacteria (15). In addition to antibacterial studies, the chemical content of different drugs of sweetgum tree was also analyzed. For example, it has been reported that sweetgum balsam contains resin, essential oil, and free acid, sweetgum oil contains high molecular weight compounds such as acid, alcohol, ester, and phenol, and sweetgum resin contains the substance "Cytorezinol".

In light of the above information, it is seen that antibacterial studies with *Liquidambar Orientalis* are generally carried out with parts of the tree such as balsam, essential oil, and leaves. However, there are only 1-2 narrow studies in the literature on tree resin. In our study, the antibacterial effect of the resin of the sweetgum tree was investigated. It is thought that this situation provides originality to the study. Also, when the studies in the literature were reviewed, it was seen that the antibacterial effect of sweetgum tree on various bacteria was investigated, but there was no comprehensive study on nosocomial infection-causing bacteria, especially. In this regard, our study will also contribute to studies of antimicrobial agent discovery for nosocomial infections.

As a result of our study determined that *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus haemolyticus* ATCC 43252, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853 bacteria exhibited different inhibition zones (between 12 and 31 mm) against the sweetgum tree resin. When the inhibition zones of the bacteria against antibiotics were compared with the CLSI 2020 standard, it was determined that *S. haemolyticus* ATCC 43252 and *E. faecalis* ATCC 29212 were resistant to vancomycin and penicillin (23). These two antibiotic-resistant nosocomial bacteria created high inhibition zones against sweetgum tree resin. So, it shows the sweetgum resin has the potential to be used as an alternative to antibiotics used against these bacteria. Similar to our study, Keskin and Güvensen investigated the antibacterial effect of sweetgum leaf extract and determined a 24 mm inhibition zone against *P. aeruginosa* ATCC 27853 strain (15). In this study, we used sweetgum tree resin and a 12 mm inhibition zone was found on the same strain. In another study, the antibacterial effect of the ethanol extract of sweetgum leaves was investigated and they found a 10 mm inhibition zone against *E. faecalis* ATCC 29212 strain (12). In our current study, it was determined that sweetgum tree resin formed a 22 mm inhibition zone on the same strain. This variability in results shows that different drugs belonging to the same plant species may have different effects on the same bacterial strain.

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Furthermore, there are also studies investigating the antifungal effect of the sweetgum tree. For example, Lee et al. investigated whether the essential oils of forty different plants, including the essential oil of the sweetgum tree, have antifungal activities on *Phytophthora cactorum, Cryphonectria parasitica, and Fusarium circinatum* species. They indicated that among all tested essential oils, sweetgum essential oil had a very high (100%) antifungal effect on *Phytophthora cactorum* (20). In another study, it was determined that the extracts prepared from *Liquidambar Orientalis* leave and resin had antifungal effects against plant pathogenic fungi such as *Botrytis cinerea* (72.96-80.65%), *Rhizoctonia solani* (10.61-28.32%), and *Alternaria solani* (27.91 - 48.8%) (21). Studies have shown that sweetgum tree also has antifungal potential. Depending on this, new studies are planned in which sweetgum tree resin will also be tested on various fungi that cause nosocomial infections (*Candida spp., Aspergillus spp., Mucorales spp., and Fusarium spp*., clinical fungal isolates, etc.). To use sweetgum tree resin as an alternative drug in the treatment of microorganisms that cause nosocomial infections, studies should be conducted with both more and different clinical isolates. In addition, there will be a need for new studies investigating the cell toxicity of sweetgum resin and its synergistic/antagonistic effects with antibiotics.

Conclusion

As a result of the study, it was seen that sweetgum tree resin has an inhibitory effect on antibiotic-resistant nosocomial infections. It is predicted that sweetgum resin may have potential use in various fields. It can be used as a medicine in the treatment of nosocomial infections, or it can be used as a disinfectant for the disinfection of medical instruments. Also, it can be used as an antibacterial film on the clothes of hospital staff or the surfaces of medical instruments.

Conflict of interest

The authors have no conflict of interest in this paper.

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