



Quercus infectoria Bark Extract Shows Antibacterial Activity Against *Staphylococcus* Species

Zainab Ahmed Aziz ^{1*}, Siham Jasim Al Kaabi ¹

Abstract

Background: *Quercus infectoria* bark is known for its various medicinal characteristics, including its phenolic compounds that exhibit antioxidant, anti-inflammatory, and antimicrobial properties. **Methods:** Fifty *Staphylococcus* spp. samples were collected from hospitals in Al-Najaf Al-Ashraf province, and *Quercus infectoria* bark extract was prepared in different concentrations. The inhibitory efficacy of the extracts against bacterial growth was assessed using the well diffusion method. Additionally, the effect of the extracts on bacterial adherence to epithelial cells was evaluated. **Results:** Both hot aqueous and alcoholic extracts of *Quercus infectoria* bark demonstrated significant inhibitory effects on bacterial isolates, with higher concentrations showing greater efficacy. The alcoholic extract exhibited superior inhibitory activity compared to the aqueous extract. Moreover, the extracts reduced the adhesion of bacterial isolates to epithelial cells, indicating their potential to prevent bacterial colonization and infection. **Conclusion:** *Quercus infectoria* bark extract showed promising inhibitory capabilities against

Staphylococcus spp. isolates and effectively reduced bacterial adherence to epithelial cells. These findings suggest the potential of *Quercus infectoria* bark as a natural antimicrobial agent for combating bacterial infections. Further research is warranted to explore its effectiveness against other bacterial species and its clinical applications.

Keywords: *Quercus infectoria*, *Staphylococcus*, Antibacterial activity, Plant extracts, Alternative medicine

Introduction

Recently, there has been a shift towards reducing the use of manufactured medicines in favor of medicinal plants due to the harmful side effects of the chemicals in manufactured medicines. Consequently, studies have explored common medicinal plants, classifying their genera and types, and identifying their natural products with medicinal, nutritional, and industrial benefits.

Quercus infectoria is notable for its biological activity, containing compounds that inhibit microorganisms, mitigate their toxic effects, and numerous other medical properties. Among the most prominent pharmacologically active components of *Quercus infectoria* bark are phenols, which are antioxidants with anti-inflammatory and antimicrobial properties (Burlacu et al., 2020). Additionally, the bark contains tannins, resins, and saponins, which are also significant. The hot aqueous and alcoholic extracts of *Quercus infectoria* bark exhibit anti-Gram-positive activity, including against *Staphylococcus* species and Gram-negative

Significance | This study determined the effect of *Quercus infectoria* bark as antibiotic and its resistance in bacterial infections.

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bacteria (Hanoon and Abd, 2021). The bark also treats diarrhea, mucosal membrane inflammation, pharyngitis, and skin inflammation. It inhibits bacterial growth by impacting quorum sensing (Deryabin and Tolmacheva, 2015).

Staphylococcus species are part of the normal flora of human skin and membranes (Lee et al., 2018). Some species are opportunistic pathogens that cause skin diseases (e.g., boils) and severe systemic diseases (e.g., pneumonia, osteomyelitis, endocarditis, enterocolitis, mastitis, cystitis, prostatitis, encephalitis, meningitis, and urinary tract infections) (Josse et al., 2017).

Given the increasing resistance of cluster bacteria to antibiotics and the difficulty of treating these infections with current antibiotics, it is essential to find natural alternatives, such as plant extracts, to combat these pathogens.

Materials and Methods

Bacterial Isolates

Fifty bacterial samples of Staphylococcus spp. were collected from hospitals in Al-Najaf Al-Ashraf province. These hospitals included Al-Hakim General Hospital, Al-Zahraa Teaching Hospital, Al-Sadr Teaching Hospital, and the Public Health Laboratory. The sampling took place from September 20, 2020, to January 10, 2021. Samples were collected from both male and female patients and included wound swabs, burn swabs, vaginal swabs, nasal swabs, urine samples from patients with urinary tract infections, and throat swabs.

Quercus infectoria Bark Collection

Quercus infectoria bark was collected from local markets in the Najaf Governorate. The bark, in small pieces of dried brown bark, was ground into a fine powder using an electric mill. This powder was then used to prepare water and alcoholic extracts in different concentrations, which were stored at -4°C until use.

Preparation of Plant Extracts

The hot aqueous extract of Quercus infectoria bark was prepared using a modified version of Harborne's method (1984) to suit the research conditions. The alcoholic extract of Quercus infectoria bark was prepared using a modified version of Al-Samarrai's method (1983), which is based on Harborne's method (1973), with adjustments made to meet the research requirements.

Inhibition Zone Measurement

The bacterial isolates were evenly distributed on Muller Hinton agar and left at room temperature for 10 minutes to allow the bacterial culture to adhere to the medium. Using a cork borer, 8 mm diameter wells were created on the surface of the agar. Different concentrations (5%, 10%, and 20%) of the aqueous extract and alcoholic extract (35, 40, 45, and 50 mg/ml) were added to the wells, with each concentration being repeated for each sample.

A positive control well containing only distilled and sterilized water was prepared, and a plate containing only the plant extract without bacterial growth was included as a negative control. The plates were then left at room temperature for 10 minutes to allow the extract to diffuse through the medium. Subsequently, the plates were incubated for 24 hours at 37°C. After incubation, the diameter of the inhibition zones was measured using a calibrated ruler (Zinedine and Faid, 2007).

Testing Bacterial Adherence

To test the influence of Quercus infectoria bark extract on bacterial adherence to epithelial cells, I adopted the method of Lomberg et al. (1989). Urine samples from a group of healthy women were collected in sterilized test tubes and centrifuged at 2000 rpm for 15 minutes. The sediment was then washed three times with phosphate-buffered saline and suspended to a volume of 2 ml per test tube. In sterilized test tubes, I mixed 0.5 ml of an 18-hour bacterial culture grown in nutrient broth, compared to a standard MacFarland tube, with 0.5 ml of epithelial cell debris as a positive control. In another set of test tubes, I mixed the same amount of bacterial culture and epithelial cells with 0.5 ml of aqueous Quercus infectoria bark extract separately. These tubes were incubated at 37°C for one hour, with intermittent shaking every 10 minutes. Phosphate-buffered saline was then added, and the tubes were centrifuged at 2000 rpm for 15 minutes to remove non-adherent cell remnants. The sediment was re-suspended in the same solution. Approximately 400 microliters of the sediment were added onto clean glass slides and allowed to air dry at room temperature. Two slides were prepared for each sample. Slides containing only bacterial culture were prepared as negative controls. The slides were stained with Gram stain and examined under an oil immersion lens to observe the attachment of bacterial cells. The attached bacterial cells were counted on each slide, and the attachment rate was calculated accordingly.

$$\text{Adhesion Rate} = \frac{\text{Total Attached Bacterial Cells}}{\text{Total Epithelial Cells}}$$

Statistical analysis

The results of the study were analyzed using the statistical program SPSS. A one-way ANOVA T-test was conducted, and the Least Significant Difference (LSD) test was used to identify significant differences between the treatments at a significance level of $P \leq 0.05$ (Morgan et al., 2004).

Results

Both the hot aqueous extract (Fig. 1) and the alcoholic extract (Fig. 2) of Quercus infectoria bark showed a significant effect on the bacterial isolates studied. When tested at concentrations of 5%, 10%, and 20% for the hot aqueous extract and 35, 40, 45, and 50

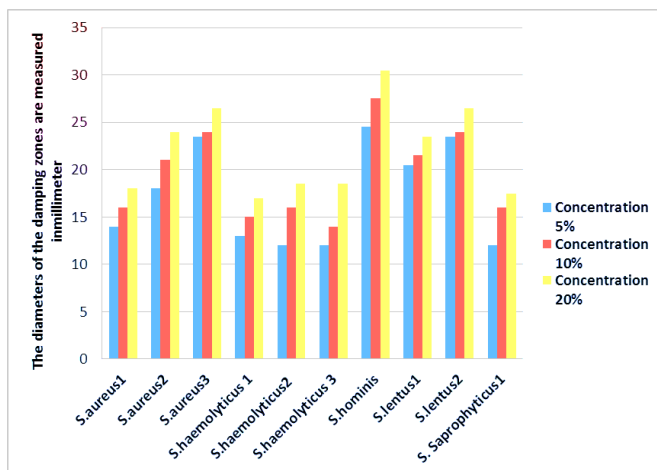


Figure 1. Effect of different concentrations of hot aqueous extract of oak bark against *Staphylococcus* spp

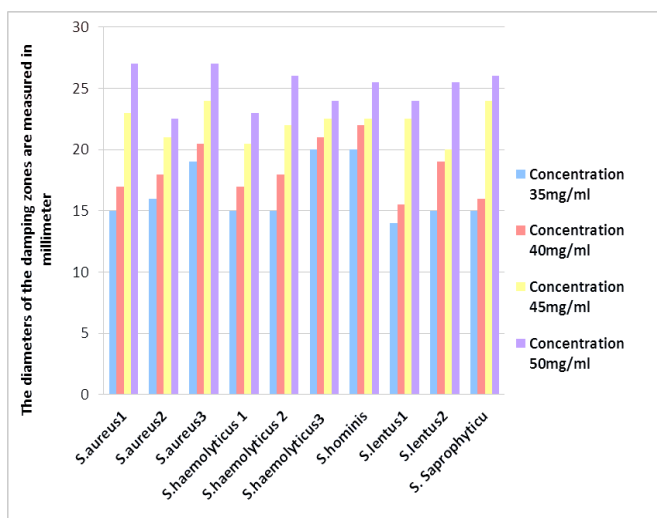
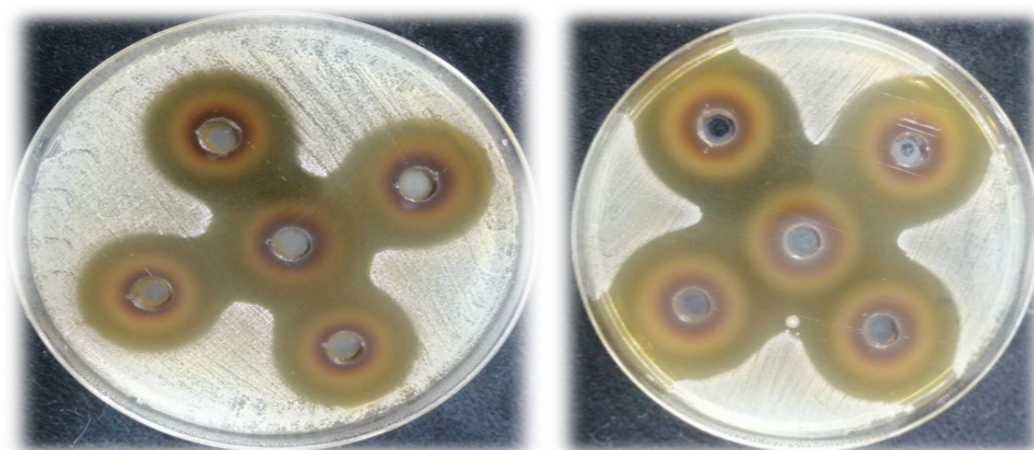


Figure 2. Effect of different concentrations of alcoholic extract of oak bark against *Staphylococcus* spp



(A)

(B)

Figure 3. Effect of oak bark extracts against *Staphylococcus* spp. **A:** alcoholic extract with a concentration of 50 mg/ml, and **B:** aqueous extract with a concentration of 20%

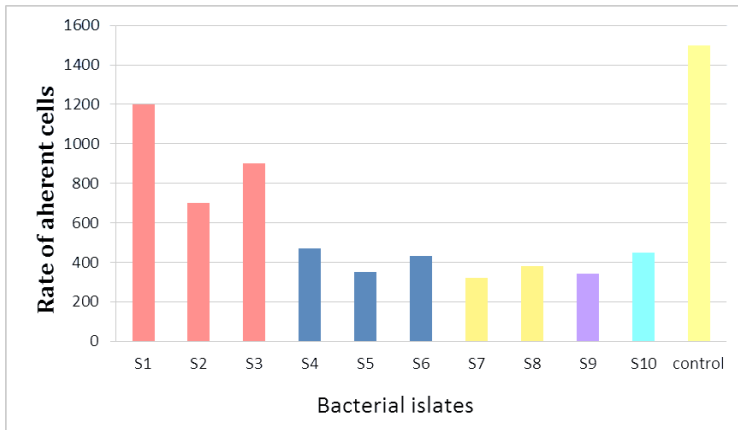


Figure 4. Variation in the rate of adhesion of bacterial isolates to epithelial cells in the presence of the hot aqueous extract compared with the control treatment.

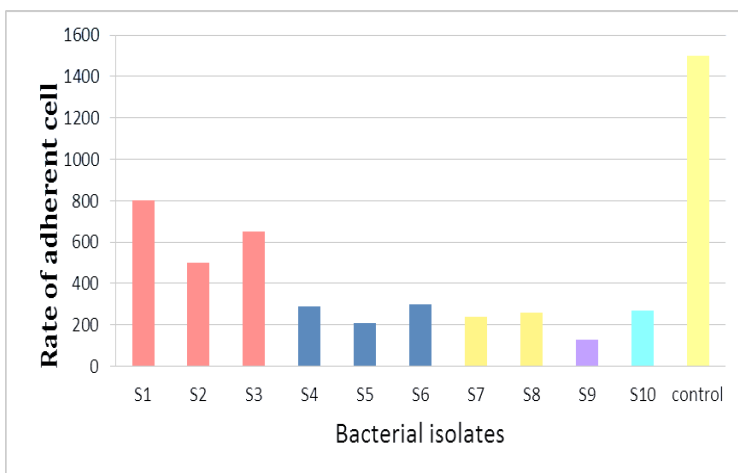


Figure 5. Variation in the rate of adhesion of bacterial isolates to epithelial cells in the presence of alcoholic extract compared with the control treatment.

mg/ml for the alcoholic extract, it was found that increasing the concentration led to an increase in the diameter of inhibition zones. The higher the concentration of the extracts, the larger the diameters of inhibition, with the alcoholic extract showing a better inhibitory effect than the hot aqueous extract (Fig. 3).

The adhesion of bacterial isolates to epithelial cells was reduced in the presence of plant extracts compared to the control treatment, which consisted of cultured bacteria with epithelial cells only. The ability of bacteria to adhere to epithelial cells in the presence of the alcoholic extract was much lower than in the presence of the aqueous extract (Fig. 4 and 5). This indicates the higher efficiency of the alcoholic bark extract in preventing bacterial adhesion to epithelial cells.

Staphylococcus aureus showed lower adhesion compared to other bacterial species, indicating its virulence. The lowest adhesion rate in the presence of the alcoholic extract was recorded for *Staphylococcus saprophyticus*, while *Staphylococcus lentus* had the lowest adhesion rate to epithelial cells in the presence of the aqueous extract.

Discussion

The inhibitory effectiveness of *Quercus infectoria* bark extract is attributed to the presence of substantial amounts of tannins, gallic acid and ellagic acid (Kokate, 1994). Tannins are phenolic compounds that dissolve in water, alcohol, and acetone, forming protein precipitates (Basri and Fan, 2005). They are considered antioxidant compounds that protect vital substances, prevent infections, and inhibit the growth of microorganisms. Tannins also have toxic effects on bacteria, fungi, and yeasts (Cowan, 1999). Additionally, phenols possess antimicrobial properties due to their mutagenic activity and impact on microbial cell membranes' permeability (Ferreira et al., 2018). They inhibit bacterial growth by suppressing enzymes responsible for protein synthesis and transformation (Newman and Cragg, 2012). The superiority of the alcoholic extract over the aqueous extract may be due to the extraction method and the type of solvent used, affecting the botanical extract's final product (Manhel and Niamah, 2012). This result was confirmed by the findings of Hanoon and Abd (2021).

Bacterial cells within their cell envelope contain different types of sugar polymers that play a key role in bacterial adhesion to host cells (Weidenmaier and Peschel, 2008). When testing the hot aqueous and alcoholic extracts of oak bark on a group of microorganisms, including *Staphylococcus aureus* and *Staphylococcus saprophyticus*, the active compounds in the extracts demonstrated antibacterial activity. This activity is related to their ability to prevent bacterial adhesion and inhibit the action of enzymes and proteins (Ray et al., 2004). Medicinal plant extracts significantly inhibit the adhesion of *Staphylococcus* spp. to epithelial cells. A study conducted by Al-Kinani (2019) showed that *Eucalyptus*

Cinnamomum camphora oil plays an important role in inhibiting the adhesion of staphylococci to epithelial cells.

It was concluded that the hot aqueous and alcoholic extracts of *Quercus infectoria* bark have inhibitory effects on the growth of staphylococcal bacterial isolates. The extracts effectively reduced the ability of *Staphylococcus* isolates to adhere to epithelial cells from healthy women. Given the proven antibacterial activity and growth inhibitory effects, further studies should investigate the effect of *Quercus infectoria* bark extract on other bacterial species.

Conclusion

In conclusion, the findings highlight the inhibitory potential of *Quercus infectoria* bark extract against the growth of the studied *Staphylococcus* spp. bacterial isolates. Additionally, the study delved into the proficiency of *Staphylococcus* spp. isolates in adhering to epithelial cells from healthy women, and explored the extract's ability to impede this adhesion efficiency. These results underscore the promising antimicrobial properties of *Quercus infectoria* bark extract and suggest its potential as a therapeutic agent against bacterial infections.

Author contributions

Z.A.A., S.J.A.K. developed the Study design, and wrote, reviewed, and edited the paper.

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Competing financial interests

The authors have no conflict of interest.

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