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An evaluation of the antimicrobial activity of some commonly used wood species: the antimicrobial effect of wood

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ABSTRACT

Aims: Antibiotic resistance mechanisms in pathogenic bacteria constitute an important obstacle in the fight against infection. Controlling the spread of resistant pathogens by utilizing antimicrobial activity on their surfaces may help us in this fight. There are many plant and wood species that have been previously tested for their antibacterial and antiviral properties. In this study, walnut (*Juglans regia*), white mulberry (*Morus alba*), white oak (*Quercus alba*), yellow pine (*Pinus sylvestris*), and beech (*Fagus sylvetica*), which are among the wood species used in the production of products that are in constant contact with humans in daily life, such as furniture, doors, and beads, were examined in terms of an antistaphylococcal effect.

Methods: Five groups of tree species and glass samples selected as a positive control were cut into 1 cm^3, contaminated with *Staphylococcus aureus* solution with a concentration of 1×10^6 CFU/ml, and monitored for five days. Each day, five randomly selected samples from each group were sonicated with phosphate buffered saline. Samples taken from the sonicated solution were cultured on tryptic soy agar (Becton Dickenson, Franklin Lakes, NJ, USA), and the number of bacteria per sample was calculated. One sample selected from the uncontaminated samples was incubated with 0.5 McFarland standard bacterial solution on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England), and the diameter of inhibition was evaluated. The study was repeated twice. The significance level was set at 0.05.

Results: The agar diffusion method showed inhibition zone only in *Q. alba* and *M. alba*. In samples contaminated with bacteria, the highest antistaphylococcal effect was found in *Q. alba*. This was followed by *M. alba*, *P. sylvestris*, *F. sylvetica*. There was no significant difference between *J. regia* and the positive control.

Conclusion: In this study in which we wanted to emphasize the importance of the antimicrobial activity of surfaces for pathogens known to live on the surface environment, all trees except *J. regia* showed antistaphylococcal effect. It is thought that the procyanidins-phenethyl, benzyl, and benzyl-in the bark of the trees are effective. In previous studies, *Q. alba* in particular is recommended for anti-infective use due to its high inhibition effect. Antibacterial activity was also found in *F. sylvetica*, which is known to have antiviral activity. Antibacterial activity was not demonstrated for *J. regia*. In order to prevent the spread of infection in collective living areas, it is recommended that trees be selected that contain antimicrobial raw materials such as *M. alba*, *Q. alba*, *P. sylvestris*, and *F. sylvetica* and to evaluate the use of extracts from these trees as natural and edible products to combat bacteria.

Keywords: Wood, antibacterial activity, antistaphylococcal efficacy

INTRODUCTION

Antibiotic resistance mechanisms of pathogenic bacteria, which are a serious risk to human health, pose a threat to treatment. Due to the genetic diversity present in bacteria, as antibiotic use increases, antibiotic resistance also increases.[1] Therefore, antibiotic use should not be accepted as the only method in the fight against infection.

Even though most of the drugs used today are synthetic, natural products, which are the primary source of traditional medicine, still constitute the main material of 25% of modern drugs.[2,3] Plants and trees can play an effective role in the fight against bacteria with the secondary metabolites they produce.[1] The aim of this study was to evaluate the antimicrobial efficacy of wood products commonly used by people in homes and hospitals, according to their species.

Environmental contamination plays a role in the spread of bacteria, especially in locations with poor hand hygiene practices.[4] Therefore, increasing hand hygiene practice and disinfection of items in contact with individuals are important steps in preventing the development of infection.[5]

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The antimicrobial activity of the materials preferred in common use areas may help in the fight against infection.

It is known that English oak bark stops bacterial activity, and onion and garlic have antimicrobial agents including ajoene and allicin.[6-8] Most of the compounds found in tree bark are thought to be involved in protection against pathogenic microorganisms in natural habitats.[9] In this study, walnut (*Juglans regia*), white mulberry (*Morus alba*), white oak (*Quercus alba*), yellow pine (*Pinus sylvestris*), and beech (*Fagus sylvetica*) were selected for examination. Since these plants are used in daily life as furniture, doors, upholstery, and rosaries, it was aimed to answer the question "Could they be potential agents in the fight against infection?"

It is known that the incidence of *Staphylococcus aureus*, which originates from skin flora and can cause infection in every tissue in the body, has increased rapidly in Western European countries in recent years.[10] Therefore, antibacterial activity against methicillin-susceptible *S. aureus* (MSSA) strains was evaluated in this study.

METHODS

Ethics committee approval was not obtained because it was an in vitro study with atcc strain. All procedures were carried out in accordance with the ethical rules and the principles.

The trees were divided into five different groups, each group containing 32 surfaces: Group 1, *Fagus sylvetica*; Group 2, *Morus alba*; Group 3, *Pinus sylvestris*; Group 4, *Juglans regia*; and Group 5, *Quercus alba*. Glass was used as control group (sixth group). Samples of 1 cm^3 were prepared, and a clean surface was obtained by wiping the samples with a sterile moistened sponge. Six different samples randomly selected from the wiped samples were inoculated into tryptic soy agar (Becton Dickenson, Franklin Lakes, NJ, USA) and checked for growth. No growth was detected. MSSA strain (ATCC 25923, used as a standard laboratory testing control strain, susceptible to a variety of antibiotics, including methicillin) was cultured, and a suspension was prepared at a 0.5 McFarland turbidity standard in sterile saline to obtain 10^8 CFU/ml bacteria.[11] For the initial evaluation, 10 microliters of bacterial stock was inoculated on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England) with sterile cotton swabs. One sample from each group was placed on the inoculated agar plate with sterile tweezers. The plate was incubated at 35±2°C for 24 hours. The diameters of the inhibition zones were measured with a ruler and photographed. Ceftriaxone (30 mcg, Bioanalyse, Gdansk, Poland) was used as control.

For the second evaluation, the final concentration was set to 1×10^6 CFU/ml by photometric measurement of the turbidity resulting from serial dilution of the inoculum prepared to a 0.5 McFarland standard. A total of 180 samples (30 samples per study group) were contaminated with 10 microliters of stock solution. Samples were stored in dark, dust-protected cabinets at 22±2°C and 55±5% relative humidity. Every day for five days, five randomly selected samples from each group were placed in 1 ml of phosphate buffered saline (PBS) solution (prepared with NaCl, KCL, Na_2HPO_4 and KH_2HPO_4), sonicated, and vortexed. One hundred microliters of the vortexed solution was cultured on tryptic soy agar (Becton Dickenson, Franklin Lakes, NJ, USA) at 35±2°C for 24 hours for colony counting. The number of bacteria per sample was calculated by multiplying the number of CFUs counted by the dilution factors. Glass samples served as positive controls. The study was repeated two times.

Statistical Analysis

The statistical package for the Social Sciences version 24.0 (IBM SPSS Inc, Chicago) was used for statistical analysis. "Minimum," "maximum." and "mean" were used for descriptive statistics. The differences between groups were analyzed by the Mann Whitney U test, and within-group differences were analyzed by the Wilcoxon signed rank test. The results were evaluated at a 95% confidence interval, and the significance level (p) was set as 0.05. Ethics committee approval was not obtained because it was an in vitro study with atcc strain.

RESULTS

Initially, in the samples tested by the agar diffusion method, an inhibition zone of 12 to 8 mm was detected around *Q. alba* and *M. alba* only ($p<0.0001$). It is shown in **Figure 1** and **Figure 2**.

Figure 1. Inhibition zone of *Q. alba*

Figure 2. Inhibition zone of *M. alba*

The growth results of 180 surfaces contaminated with bacteria according to groups and days and p values compared to the control group are given in Table 1. The highest growth was detected in the control group on all days. There was no difference between the amount of growth detected in the *J. regia* samples and the control group on any day (p>0.05). *Q. alba* was the only tree in which no growth was detected from the first day. Antimicrobial properties were detected in *Q. alba*, *M. alba*, *P. sylvestris*, and *F. sylvetica*, respectively. The antimicrobial activity of *F. sylvetica* was significantly lower than the others (p<0.001).

DISCUSSION

It is known that pathogens can survive and spread in surface environments.[9] In this study, we evaluated the potential antimicrobial properties of *P. sylvestris*, *F. sylvetica*, *J. regia*, *Q. alba*, and *M. alba*, which are particularly used in the wood industry. The antibacterial properties of various wood and bark extracts have been previously described.[1,8,12-14]

To evaluate antimicrobial activity, MSSA bacteria, which can cause widespread systemic infection particularly from skin colonization, were selected. In addition to *J. regia*, MSSA was also susceptible to *P. sylvestris* and *F. sylvetica*, especially *M. alba* and *Q. alba*. Other studies involving wood and bark have also shown that Gram-positive bacteria are susceptible, and their growth is inhibited.[15] The reason for this may be that procyanidins in wood and bark form complexes with DNA, or phenol compounds inhibit protein kinases including DNA gyrase.[16,17]

It has been previously shown that *Q. alba* exhibits antibacterial activity against Staphylococcus epidermidis.[18] In another study, the use of *Q. alba* in wound healing of staphylococcal infections in case of resource scarcity was recommended.[1] In our study, the results of previous studies for *Q. alba* were supported.

Table 1. Reproduction amounts detected in groups by days

	Fagus sylvetica (CFU/ml)	*Morus alba* (CFU/ml)	*Pinus sylvestris* (CFU/ml)	*Juglans regia* (CFU/ml)	*Quercus alba* (CFU/ml)	Control (CFU/ml)
First Day						
Min	1000	0	400	2300	0	2500
Max	2000	30	800	4000	0	4000
Mean	1460	10	580	2770	0	3040
p	<0.001	<0.001	<0.001	0.143	<0.001	
Second Day						
Min	200	0	200	600	0	600
Max	900	0	500	1100	0	1000
Mean	460	0	330	830	0	730
p	<0.001	<0.001	<0.001	0.123	<0.001	
Third Day						
Min	100	0	30	300	0	300
Max	250	0	80	700	0	700
Mean	155	0	51	440	0	410
p	<0.001	<0.001	<0.001	0.436	<0.001	
Fourth Day						
Min	60	0	10	300	0	200
Max	120	0	30	500	0	600
Mean	89	0	19	340	0	320
p	<0.001	<0.001	<0.001	0.393	<0.001	
Fifth Day						
Min	60	0	10	100	0	100
Max	100	0	20	200	0	300
Mean	80	0	13	130	0	170
p	<0.001	<0.001	<0.001	0.353	<0.001	

Previous studies with the *Pinaceae* family have also shown that it has gram-positive activity compared to tetracycline.[19] In our study, the antistaphylococcal activity of *P. sylvestris* was confirmed.

It is known that the phenethyl, benzyl, and benzoyl groups contained in *M. alba* show antimicrobial activity by crossing bacterial membranes.[20] In our study, *M. alba* supported this result by showing high antimicrobial activity.

The antiviral activity of 40-Me-glucuronoxylan sulfate contained in *F. sylvetica* against HSV-1 and HSV-2 has been shown previously.[21] In our study, antibacterial activity was found to be lower than other wood species but significantly different from the control group.

It has been shown that extracts made from the leaves and stem of *J. regia* have antibacterial activity for Gram positive bacteria, especially staphylococcal antimicrobial activity and antifungal activity.[22] However, the fact that there was no difference between *J. regia* and the positive control group in our study suggests that it has no superficial activity and gains activity as a result of chemical processes.

Study Limitations

However, the limitation of this study is that we do not have information about the age and chemical content of the wood. The effect of oiling and varnishing used to prevent bedbugs on some types of furniture used in daily life could not be evaluated. Since the effect on other Gram-positive pathogens could not be evaluated, the study only measured antistaphylococcal activity. This study should be considered only as a preliminary analysis of antimicrobial activity, since microdilution, which is accepted as a reference method, was not used. The actual susceptibilities can be determined after microdilution using the sap of the trees.

CONCLUSION

Our findings showed that *M. alba* and *Q. alba* have a strong antimicrobial effect on the growth of *S. aureus*, an important pathogen, while *F. sylvetica* and *P. sylvestris* have a relatively weaker antimicrobial effect. In order to prevent the spread of infection in public living areas, it is recommended that furniture and furnishings be used whose raw materials have antimicrobial properties. The use of wood bark, which is separated as waste and by-product in industrial processes, for antimicrobial activity also increases the use of natural and renewable products.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval was not obtained because it was an in vitro study with atcc strain.

Informed Consent: Informed consent is not required.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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