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Antimicrobial activity and influence of wood extractives concentration on the heritage wood properties

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Abstract

Developing new eco-friendly wood preservatives resistant to fungal and bacterial attacks is a primary industrial goal. Wood extractives play a crucial role in wood protection against microbial deterioration. This study investigated the impact of wood extractive concentration on heritage wood properties. An experimental wood sample of the same species of heritage wood (Pinuspinea L) has been selected to run the investigation before and after exposure to heat at 100 °C for 48 hours to mimic the wood aging that has taken place over many years. Extraction was performed using different solvents (benzene/methanol, ethyl acetate, n-hexan, and distilled water). Chemical composition, morphological structure, and thermal degradation were investigated by infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and thermal gravimetric analysis (TGA) techniques. The mechanical properties of wood have been studied before and after heat treatment to determine the impact of extractive shortages on wood properties. Additionally, the antimicrobial activity of wood extractives was investigated against Gram-positive bacteria, Gram-negative bacteria, yeast, and fungal test strains.

Keywords: Heritage Wood; Extractives;Lignocellulosic Material; Antimicrobial Activity; Heat Treatment

1. Introduction

Conservation and protection of historical wood has excellent research interest due to its great value to countries. It represents their history and civilization. Wood is a complex material considered one of the most available resources in the bio-based industry. It is the most widely used material in human history for producing artistic works manufactured from wooden objects. It is known as archaeological wood, as described by archaeologists in the historical era. It comprises lignocellulosic materials such as cellulose, hemicelluloses, lignin, and extractives, which are collected together physically and chemically to stimulate its use in many applications, particularly in cultural heritage [1-4]. However, wood has been threatened by many damage factors, such as bacteria and fungi that attack with longevity from outside exposure. Inhibition of microbial growth in the wood returns to the presence of extractives that are non-structural components in its heartwood [5-7]. Although these extractives represent a minor fraction in wood, they protect against bacterial and fungal attack. These extractives have two main compounds: i) aliphatic compounds such as resin, and ii) phenolic compounds such as simple phenol and lignans. It comprises fats, resin, and wax that vary according to the part of the tree used for the isolation [8, 9]. Cellulose is the central part of the wood, which is β(1→4) linked D-glucose units [10]. It is easily insoluble in conventional solvents owing to its intra- and inter-molecular hydrogen bond formation. Thus, it is solid and resistant to hydrolysis. Scientists have extensively studied it over decades [11, 12]. The second component of wood is hemicelluloses, which

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consist of heteropolysaccharides. It plays a vital role in the bonding capacity of fibers and provides plant fiber network strength. However, hemicelluloses have a random, amorphous structure with little strength and are readily hydrolyzed by dilute acid or base [13, 14]. Lignin is a highly complex three-dimensional polymer surrounding cellulose and hemicellulose that strengthens the cell walls' rigidity and, consequently, the whole plant structure. It also prevents the penetration of chemicals that can break the cell wall and protects the plant against microbial attack [15, 16].

The present study aims to understand the effect of the extractives' concentration on the wood and its properties due to exposure to heat treatment at 100 ℃ for 48 h, miming wood aging for many years. An experimental wood sample of the same species of heritage wood (*Pinuspinea L*) is selected for this investigation. Wood's chemical and morphological structure was characterized before and after the heat treatment by FTIR, SEM, TGA, and XRD analysis. The antimicrobial activity of the extractives against different test microbes belonging to other groups was also evaluated.

2. Experimental

2.1. Materials

The heritage wood is an archaeological wooden door of KhawandAsalbay Mosque, which is also mistakenly referred to as that of "Qaytbay Mosque" in Fayoum, which was built over the BaherYousaf canal. The measurements of the wooden door are 3 m in length and 2 m wide [17]. The experimental wood (*Pinuspinea L*) was obtained from Egypt's wood market. The chemical compositions of the practical wood have been determined, including hollow cellulose, lignin, and ash content. The results were (80%, 17%, and 0.4%), respectively, according to Tappi standards [18-20]. All chemical reagents were used without further purification.

2.2. Heat treatment of wood

Wood samples with 10 x 10 x 1 cm dimensions were prepared for bending tests. While samples with dimensions of 2 x 2 × 2 cm were prepared for compression tests, they were placed in an oven, and the temperature was increased by 20 ℃ min-1 from ambient to the operating temperature of 100 ℃. All samples were weighed before and after heat treatment [1]. The loss percentage of the mass has been calculated by calculating the weight of the samples before and after the heat treatment and recording the results. The rate of loss in the density of wood was calculated by applying the density law [21] as in equation (1).

$$\boldsymbol{Density} = \frac{\textbf{mass (kg)}}{\textbf{volume } (\boldsymbol{m^3})} \qquad \textbf{(1)}$$

Heat treatment has taken place in the LABTECHkiln oven at the National Research Center, Giza, Egypt.

2.3. Extraction of extractives

Different solvents (benzene/methanol, ethyl acetate, n-hexan, and distilled water) were used to find out what wood extractives were present before and after heat treatment. The Soxhlet apparatus typically uses selective organic solvent extraction to determine these products. 20g of air-dried wood and 1L of solvents were extracted for 18 hours. The extracted substance was air-dried and gravimetrically calculated [5, 22].

2.4. Mechanical properties

Before and after heat treatment, the ASTM D638-91 standard was used to measure the bending and compressive resistance. A universal testing machine LK10k (Hants, UK) with a 5 kN load cell was used. The operation was done at a 5 mm/min rate on the samples cut to suit the machine's operating conditions. The unit of measurement is (Newton) [23, 24] as in equation (2).

$$\boldsymbol{Modulus\ of\ rupture(\ MOR)\ for\ bending\ test} = \\ = \frac{\textbf{3}*\textbf{length(mm)}*\textbf{Max.Load (N)}}{\textbf{2}*\textbf{width(mm)}*\textbf{thickness (mm)}} \qquad \textbf{(2)}$$

2.5. Physico-chmical properties

- *Infrared (IR) spectral analysis*

FT-IR spectra of wood before and after heat treatment were recorded in the range of 400–4000 cm^{-1} on the Shimadzu 8400S FT-IR Spectrophotometer.

- *Scanning electron microscopy (SEM)*

The surface morphology of wood before and after heat treatment was analyzed using an electron microscope (FEI IN SPECTS Company, Philips, Holland) and environmental scanning without coating.

- *X-ray diffraction*

The XRD patterns of the experiment wood before and after heat treatment were detected using an X-ray diffractometer. X-ray diffraction patterns were

obtained using a Bruker D8 Advance X-ray diffractometer (Germany). A copper (Kα) target and a secondary monochromator set to 40 kV and 40 mA were used to record the diffraction patterns.

- *Swellingbehavior*

The swelling behavior of wood before and after heat treatment was monitored gravimetrically. This experiment immersed wood samples in distilled water at room temperature for 24 hours. The swollen piece was removed, pressed between two filter papers to remove the excess water, and weighed. The swelling percentage was determined through different time intervals, as in equation (3) [25].

$$\textbf{\textit{Swelling}}\ \% = \frac{(m_t - m_0)}{m_t}\ \textbf{\textit{X}}\ \mathbf{100} \qquad \textbf{(3)}$$

Where m_t is the weight of the swollen wood sample at time t and m0 is the weight of the dried wood sample.

- *Thermo gravimetric analysis*

The thermal stability of wood before and after heat treatment was carried out using a TGA Perkin-Elmer (STA6000) with a heating rate of 10 ℃/min. The temperature ranged from room temperature (30 ℃±5) to 800 ℃ under atmospheric conditions (10 ℃/min).

- *Antibacterial Activity*

An agar plate method was developed to evaluate the antimicrobial activities of the extractives. The four representative test microbes used were *Staphylococcus aureus* ATCC 6538-P (G+ve) and *Escherichia coli* ATCC 25933 (G-ve), *Candida albicans* ATCC 10231 (yeast), and *Aspergillusniger* NRRL-A326 (fungus). The bacterial and yeast test microbes were grown on a nutrient-agar medium. On the other hand, the fungal test microbe was cultivated on a Czapek-Dox medium. The culture of each test microbe was diluted by distilled water (sterilized) up to 107 or 108 colony-forming units (CFU/ml), and then 1 ml of each was used to inoculate a 1L Erlenmeyer flask containing 250 ml of solidified agar media. These media were put on previously sterilized Petri dishes of 10 cm diameter with 25 ml of hardened media. Extractive discs (10 mm in diameter) were loaded and placed on the surface of the agar plates, seeded with test microbes, and incubated for 24 h at the appropriate temperature for each test organism. Antimicrobial activities were recorded as the diameter of the clear zones that appeared around the extractive sample [26-28].

3.Results and discussions

3.1. Characterization of the heritage wood

Figure 1a, b shows the surface morphology of the heritage wood under a scanning electron microscope and a lighting microscope. The surface appears rough with a high porosity structure due to the loss of the extractive content, which decreases its physical and chemical properties [24, 29].

Figure 1c represents the FTIR of the heritage wood. The main components of the samples are cellulose and lignin. Cellulose has characteristic peaks of OH stretching at 3500 cm^{-1}, CH stretching at 2900 cm^{-1}, and ether linkage of C-O-C at 1047 cm^{-1}. The peak appeared at 1700 cm-1, attributed to a phenolic compound of lignin [23, 27, 30].

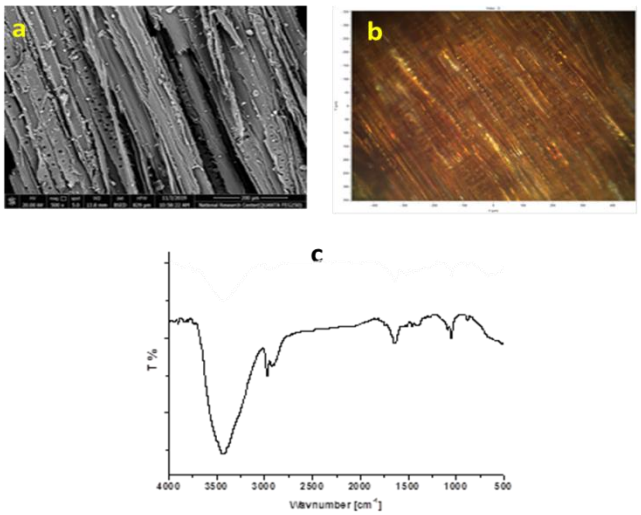

Fig. 1: a) Scanning electron microscopy, b) lighting microscope, and c) FTIR of Heritage wood.

3.2. Characterization of the experimental wood

To study the impact of extractive concentration on the heritage wood, we selected an experimental wood sample of the same species (*Pinuspinea* L). Then, the observed sample was exposed to 100 for 48 hours to achieve a high degree of statute of limitations. After exposure to 100 for 48 hours, the wood became fragile and breakable due to decreased durability and extractives that joined the matrix chain.

- *Determination of extractives*

Wood contains varying amounts of extractives composed of non-structural components such as resins, fats, oils, and additives. Thus, according to their polarity, different solvents were used (benzene/methanol, ethyl acetate, n-hexan, and distilled water). The benzene/methanol system was more effective than other solvents due to its high polarity and ability to dissolve the extractives. The extraction process has occurred before and after the

heat treatment. Table 1 shows how heat treatment and volatilization reduce extractive quantity. This loss in extractives affected the other properties of wood. Modulus of rupture (MOR) represents the mechanical properties of wood, bending, and compressive resistance related to the behavior of wood material when exposed to heat or pressure [24]. Where MOR decreased when the wood extractives decreased, which bound the wood matrix, the density of the wood decreased because of heat treatment due to the mass loss. The swelling of the wood increased due to cracks and holes formed by heat treatment that appear on the wood surface, as in the optical photo in Figure 3. Additionally, the physical properties of wood, such as color, change after heat treatment from off-white to dark brown, and the samples become fragile [1].

Table 1: The properties of the experimental wood before and after heat treatment

Test	Before	After
Extractives %	5	2.3
Modulus of rupture(MOR)N/mm^2	91.6	79.3
Swelling (g/g)	45	51
Density (g/m^3)	0.7	0.6

- *Scanning electron microscopy (SEM)*

Figure 2 displays the effect of heat treatment on the wood surface morphology. The surface of the wood before heat treatment appears as a compacted surface due to the fibers binding to the extractive adhesive properties. The surface of wood after heat treatment appears clearly fragile and breakable, with cracks and holes on the surface due to the loss of adhesive extractives that joined the plant cells together [31].

- *Infrared (IR) spectral analysis*

FTIR analysis is a valuable tool to confirm the wood's chemical structure. Figure 3 illustrates the FTIR analysis of wood samples after and before heat treatment. The main components of wood are cellulose and lignin, where its characteristic peaks appear clearly. Cellulose showed absorption bands of OH stretching at 3000–3500 cm^{-1} and CH stretching at 2900 cm^{-1}[29, 32]. The peak at 1640 cm^{-1}represents the OH bending of the adsorbed water in cellulose [27]. After heat treatment, degradation and deformation occurred in the cellulose chain, so the wood became fragile, breakable, and lost its durability. It appears in the adsorption band of ether linkage C-O-C at 1047 cm^{-1} that joins the cellulose chain together; it decreased and became so small after the heat treatment.

As well, the peak at 1450 cm^{-1} of cellulose crystallinity also decreased after heat treatment [26, 27]. The distinctive peak of phenolic compound C=O appears at 1742 cm^{-1} , also became small after the heat treatment[9, 33].

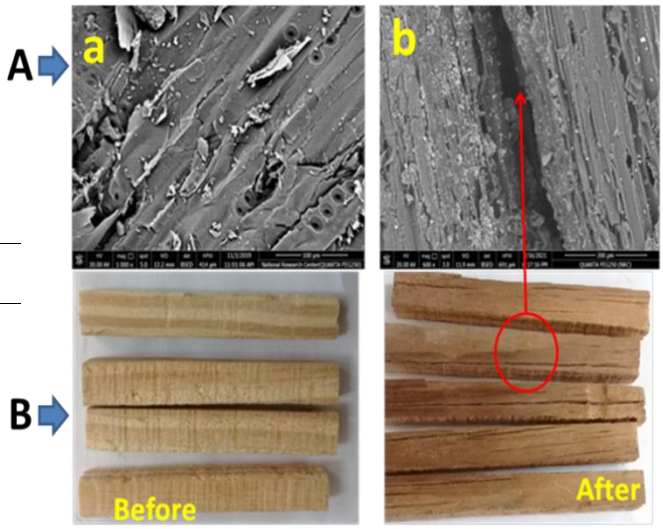

Fig. 2: A) SEM of the experimental wood a) before, b) after heat treatment.

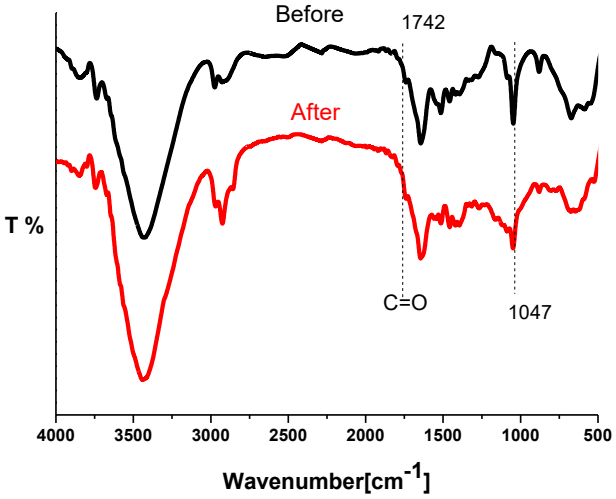

Fig. 3:FTIR of the experimental wood before and after heat treatment.

- *X-ray diffraction*

The cellulose pattern shows two peaks at 2θ = 15°and 22°, which represent cellulose I [11]. Figure 4

shows the X-ray diffraction pattern of wood after the heat treatment process; the peaks at this region increased and became sharper after heat treatment due to cellulose degradation and decreased extractive concentration, which makes an adhesive and joins the fibers together [34, 35]. It also appears that the crystalline region at (2θ) =15 increased more after heat treatment than before.

- *Thermo gravimetric analysis*

Figure 5 displays the wood's thermal gravimetric analysis curves before and after heat treatment. The thermal stability behavior of wood highly differs before and after heat treatment due to a decrease in its durability and weak properties, so the wood becomes fragile and breakable. Before heat treatment, the wood was stable at 250 ℃ and started to decrease at 300 ℃ due to moisture evaporation [36]. By increasing the degradation temperature to 400 ℃, it lost about 25% of its initial weight, leaving the remaining residual of about 75%. After heat treatment, the wood started to decrease at 250 ℃, and by increasing the degradation temperature to 350 ℃, it lost about 70% of its initial weight, leaving the remaining residual about 30%. The degradation process started faster in wood exposed to heat treatment than in wood not exposed to heat treatment. This behavior is attributed to decreasing the crystallinity and deformation of the wood [11, 15].

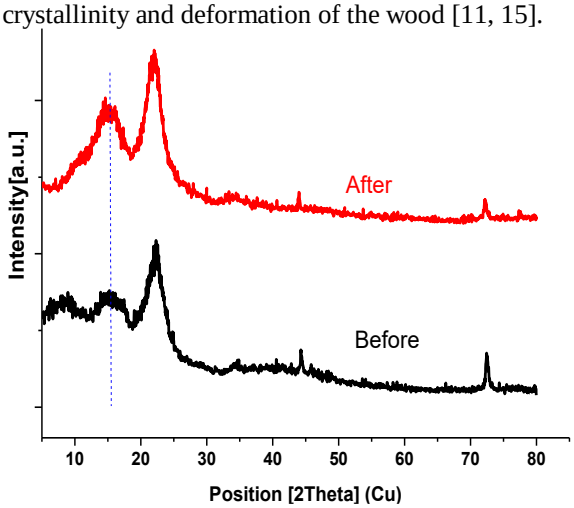

Fig. 4: X-ray diffraction of the experimental wood before and after heat treatment.

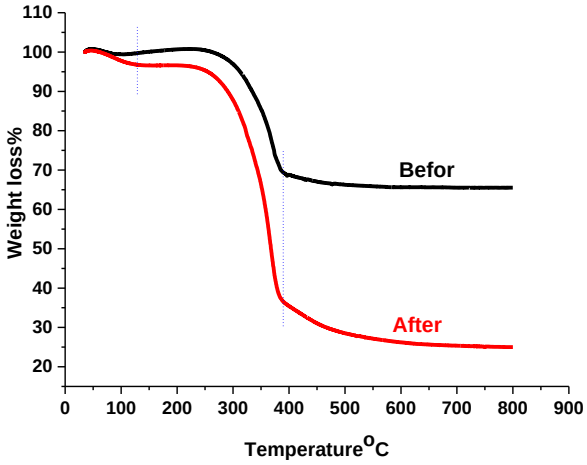

Fig.5:TGA of the experimental wood before and after heat treatment.

- *Antibacterial Activity*

The current study looked at how well the extracts killed gram-positive (*Staphylococcus aureus*), gram-negative (*Escherichia coli*), yeast (*Candida albicans*), and fungus (*Aspergillusniger*) bacteria using the agar plate method. The results showed that the extractives have higher activity against *S. aureus*, *A. niger*, and *C. albicans* than *E. coli*. The clear inhibition zone shows the antimicrobial activities of the extractives are high against gram-positive, gram-negative, and fungus (Table 2). Antimicrobial activity is due to reactive oxygen species (ROS), which are produced by phenolic compounds in the extractives that react with the thiol-SH group in proteins to form H_2O_2, and forming radicals can destroy the membrane of the microbe [23]. It may also be due to hydrogen bond formation between phenolic compounds and bacterial proteins. This interaction damages the cytoplasmic membrane and destroys the protein to prevent the nutrient uptake process in the bacterial cell [37, 38].

4.Conclusion

This work demonstrates the effect of the extractive's concentration change on the wood's properties. An experimental wood sample of the same species of heritage wood (*Pinuspinea* L) has been selected successfully and exposed to heat treatment for 48 hours to mimic the sample. It has been investigated via SEM, XRD, TGA, and FTIR. The wood extractives were measured using different solvents. The results showed that the presence of extractives significantly impacts wood properties. It enhances its physical and chemical properties and protects against microbial attack due to its antimicrobial activity against Gram-positive, Gram-negative, yeast, and fungal test microbes.

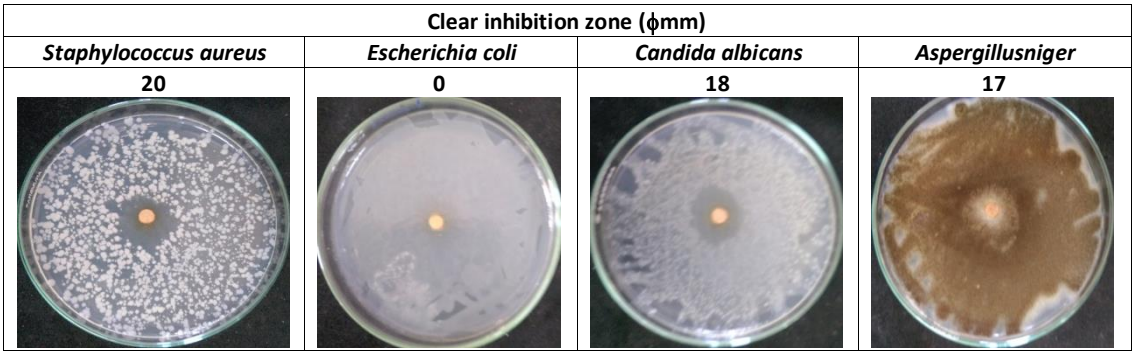

Fig. 6: The inhibition zone of Antimicrobial activity of the experimental wood extractives.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgments

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7. References

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