ANTIMICROBIAL PROPERTIES of WOOD WATER OBTAINED by A HIGH FREQUENCY VACUUM DRYING METHOD

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Abstract: The objective of this study was to determine the antimicrobial activity properties of the wood water released from specimens during the drying process. The specimens were dried using a high-frequency vacuum (HfV) drying method. Wood specimens from white oak (*Quercus alba* L.), walnut (*Juglans regia* L.), and Eastern beech (*Fagus orientalis* L.) were dried inside a drying kiln with settings of 7.56 MHz frequency, 39.19λ wavelength, and 0.5 to 0.97 mBar vacuum. The moisture content (%) of the white oak, walnut, and Eastern beech specimens prior to kiln drying were 70% to 75%, 80% to 90% and 75% to 80%. Drying days of wood specimens were 16, 10, and 8, respectively. The antibacterial and antifungal activities of the wood water samples were determined using the microdilution method. According to the antimicrobial test results, the best antimicrobial properties were found in the white oak's drying water, with only 6.3 mg/mL of K. *pneumoniae* bacteria.

Keywords: Antibacterial activity; antifungal activity; wood water; high-frequency vacuum (HfV) drying

Introduction

Drying is the process to reduce water of wood to make wood material ready for lots of areas of usage. And, also drying is important for potential use of obtained water while drying process (Altınok et al. 2009). Drying methods are classified into two; natural and technical. In the past, wood materials were dried naturally using the sun's radiation and air flow; however, technical methods of drying are currently employed. Technical drying includes many different methods, which have come about through the advances in technology. The most common industrial drying methods for solid wood materials are classical, condensation, and vacuum drying. In addition, high-temperature, high-frequency, and microwave drying methods are less commonly applied. The first industrial radio-frequency vacuum (RfV) driers were developed by the Russian Academy of Sciences in the 1960s. High-frequency vacuum (HfV) driers were developed by Koppelman in the USA in 1967 (Resch, 2006). Currently, the HfV combined drying method prevails because of its many advantages in comparison with classical drying methods. Classical drying methods are insufficient for drying thick lumber without any deformation and also take longer without achieving the desired quality level (Güler et al. 2012).

Wood properties, such as combustibility, color, density, smell, feel/sense, compressive strength resistance, and dehumidifying capability, vary depending on the amount of extractives present in the material (Tutuş et al. 2010). Wood extractives evaporate while drying process because there is an open drying system either natural or technical drying is used. However, HfV is a completely closed drying system that does not require a chimney or additional humidifying equipment. Therefore, the water released from the drying process is generally purer because there is not any mixture. Therefore, utilization of obtained water which is rich in terms of extractives becomes meaningful (Güler et al. 2012). The released wood water can be further processed and utilized in cosmetic goods such as perfume and creams because of its pleasant aroma.

Turkey has an abundance of flora in terms of plant diversity. Turkey is located on the border of three phytogeographical regions and is a bridge between South Africa's and Southeast Asia's flora. In addition, species endemism is high in Turkey because Anatolia is the origin and differentiation center of many kinds and section. These can be assumed as some of the reasons of this abundance. There are approximately 9000 natural plant species and 30% of these are endemic, but still not enough benefited from the richness of this plant (Tan, 1992). Plants' antimicrobial activities and properties are important for human health and have been investigated since 1926 (Vonderbank, 1949; İlçim et al. 1998). According to the World Health Organization's research, the number of medicinal plants for therapeutic purpose is around 20,000 (Dığrak et al. 1999). Increased incidence of drug resistance which has become a global issue in recent years is reported because of the adaptations of certain bacteria species to antibiotic strains (Kalaycıoğlu and Öner, 1994). This problem has a great importance in developing countries because of an excess in the number of reported infectious diseases (Abascal and Yarnell, 2002). This is why the use of medical plants as an alternative to synthetic medicine (e.g. penicillin) is recommended and plants are being introduced as antimicrobials (Solanki, 2010). Currently, medical plants are utilized because they exhibit less adverse effects and microbial resistance than synthetic medicines that are already in use. However, medical plants may provide a slower recovery rate (Seyyednejad and Motamedi, 2010). Results of some research studies show that the antimicrobial agents obtained from natural resources, i.e., plants, highly succeed in protection of food safety (Alzoreky and Nakahara, 2003). According to the literature, antimicrobial activity occurs in the presence of secondary metabolites inside of a plant, such as alkaloids, flavonoids, tannins, and terpenoids. Secondary metabolites which are not directly related to the basic vital function of the plant are produced in woody plants. Furthermore, these secondary metabolites consist of herbivores and pathogens to provide protection from abiotic surrounding stresses, e.g., UV radiation (Toroğlu and Çenet, 2006). Secondary metabolites are a diverse group that includes many compounds. Phenolic compounds are extremely important because of their role in anti-carcinogenic and antimicrobial effects. Also, it is known that phenolic compounds exhibit a potent antioxidant effect by quenching free radical activity. Free radicals can cause damage to tissues and DNA by attacking the nucleic acids, somatic cells, and weakening the immune system (Zhao et al. 1999; Bouwmeester, 2003).

In light of this information, the aim of this study is to determine the antibacterial and antifungal properties of water obtained using the HfV drying method. And, also remark the utilization possibility of obtained water in different sectors.

Materials and Methods

Three tree species, i.e., white oak (*Quercus alba* L.), walnut (*Juglans regia* L.), and Eastern beech (*Fagus orientalis* L.), were used in this study. White oak was grown in small plantations on the stream beds and slopes in Thrace, Marmara, and Northwest Anatolia regions of Turkey. Oak logs were 15 to 21 cm thick, 30 cm wide, and 300 to 350 cm length and had 70% to 75% inlet moisture. Walnut logs were generally grown in the East Anatolia region of Turkey (Adıyaman, Muş, Bitlis, and Bingöl environments). Walnut logs were in the form of gunstock and rifle butt and had dimensions of 7 to 7.5 cm thick, 16 to 20 cm wide and 45 to 90 cm length, as well as 80% to 90% inlet moisture. Eastern beech logs had various dimensions and 75% to 80% inlet moisture.

In this study, following bacteria, yeast and fungi were used to investigate antimicrobial activity. *Staphylococcus aureus* (6538P), *Klebsiella pneumoniae* (CCM 2318), *Escherichia coli* (ATCC 11230), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 6897), *Bacillus cereus* (CCM 99), *MRSA* (ATCC 33592), *Salmonella typhimurium* (ATCC 14028) were bacteria. *Candida albicans* (ATCC 10239) was yeast. *Aspergillus niger* van Tiegh (TA 47-3), *Aspergillus flavus* Link (TA 41-17), *Aspergillus ochraceus*, K. Wilh (MUCL 39534), and *Fusarium proliferatum* (Matsushima) Nirenberg (TA-18-2) were fungi.

This study was conducted in two stages. In the first stage, water released from the wood logs and collected in a barrel using the HfV drying method as shown in Fig. 1. A 25-m3 drying kiln (King Dryer, HF+V 5025, Düzce, Turkey,) was used for the drying processes, with the parameters of 7.65 MHz frequency, 39.19λ wavelength, and 0.5 to 0.97 mBar vacuum.

After the drying process, the moisture content (%) of the oak, walnut, and beech logs were between 8.0% and 15%, 8.0% and 15%, and 7.0% and 8.0%, respectively. The drying durations of the oak, walnut, and beech logs were 15 to 17 (avg. 16) days, 8 to 11 (avg. 10) days, and 7 to 9 (avg. 8) days, respectively. The obtained wood water was kept fresh (not ex-stock) and was protected from direct sunlight.

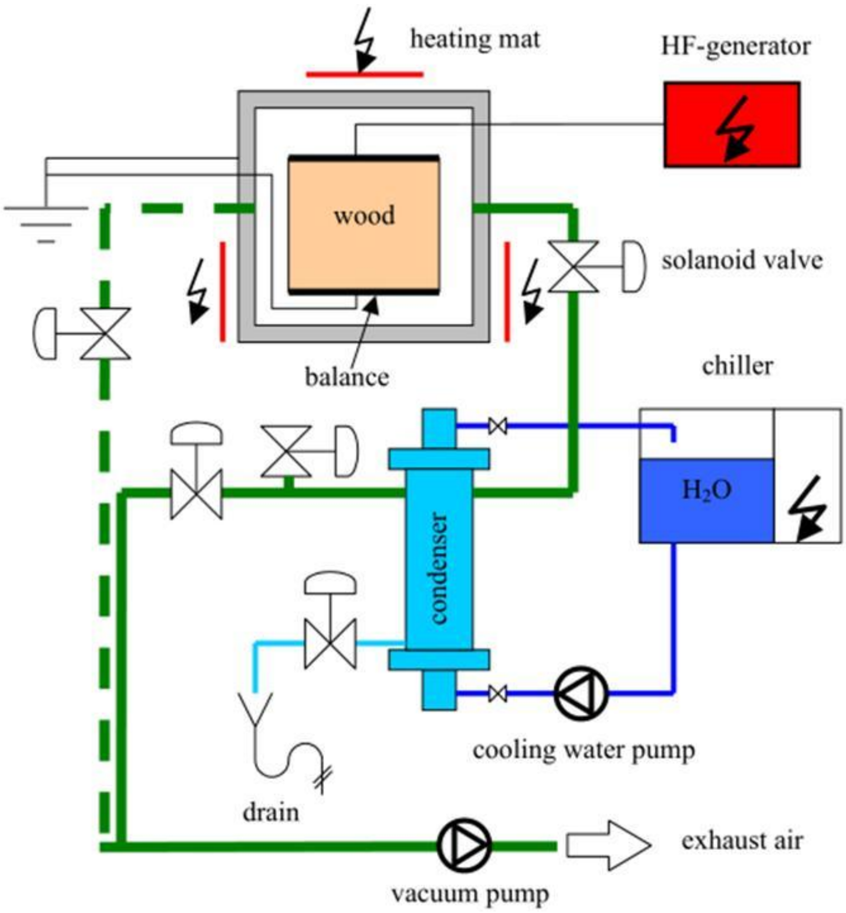

Figure 1: Schematic of the high-frequency current vacuum dryer (Korkut, 2011; Resch and Hansmann, 2002).

In the second stage of this study, the microdilution method was used to determine the antimicrobial activity. Bacteria suspensions were prepared according to the normal saline and MacFarland 0.5 standards for antibacterial activity. Also, trueness of the measurements was confirmed at a 0.5 absorbance value read from the spectrophotometer's indicator at 600 nm. Fungus inoculum activity was adjusted at a 0.5 absorbance value read at 450 nm. Bacteria and fungus were subjected to incubation on nutrient agar slant at 37°C for 24 h and Malt agar slant at 28°C for 72 to 96 h, respectively. Inoculum suspensions were prepared with fresh cultures and studied in three series on well-plates for each microorganism. Sabouraud Dekstroz Broth (Oxoid Ltd., Hampshire, England) and Nutrient Broth (Merck KGaA., Darmstadt, Germany) were used as the medium for the fungus and bacteria, respectively. At first, 100 µL of medium was pipetted into all wells, expect the first one. And after, 150 µL of medium and 50 µL of extract were pipetted into the first well and dilutions were serially made by taking 100 µL of the extract solution out from the previous wells (from 2nd to the 11th well). The final extraction concentration in the wells was prepared between 0.05 mg/mL and 25 mg/mL when the dilution processes were completed. Exactly 20 µL of inoculum suspension was grafted into all wells after the dilution process except negative control. All of the microplates were incubated at 37 °C for 24 to 48 h for bacteria and 28 °C for 72 h for fungi. After the incubation period, concentration which had higher value and was prior to the growth realized concentration was recorded as the minimum inhibitory concentration (MIC) values (CLSI, 1997; Walsh, 2008). The MIC values were expressed as the static activity of microorganisms, or in other words, expressed as the concentration value where the growth of microorganisms is inhibited. However, the bactericidal and fungicidal terms express the concentration value which kills the microorganism. In this method, 20 µL was extracted from each well plates' wells (no growth ones) and then cultivated by applying to petri plates of Sabouraud Dekstroz Agar for fungus and Nutrient Agar for bacteria.

The values of growth unrealized concentrations denoted the minimum bactericidal concentration (MBC) for bacteria and the minimum fungicidal concentration (MFC) for fungus after the incubation process under the above-stated conditions (CLSI, 1997).

Results and Discussion

MIC values which inhibit the growth of the microorganism and MBC and MFC values which represent the values of bactericidal and fungicidal activity were presented in Table 1.

Table 1: MIC and MBC/MFC Values (mg/mL) of Wood Water Samples.

Microorganisms	Oak (*Quercus alba* L.)		Beech (*Fagus orientalis* L.)		Walnut (Juglans regia L.)	
	MIC	MBC	MIC	MBC	MIC	MBC
P. aureginosa	6.3	12.5	12.5	12.5	12.5	25
S. aureus	6.3	12.5	12.5	12.5	6.3	12.5
K. pneumoniae	1.6	6.3	6.3	12.5	12.5	>25
P. vulgaris	12.5	>25	12.5	>25	12.5	>25
B. cereus	12.5	>25	12.5	25	25	>25
E. coli	25	25	12.5	>25	25	>25
MRSA*	12.5	25	12.5	>25	12.5	25
S. typhimurium	6.3	>25	25	>25	25	25
C. albicans	12.5	25	25	>25	12.5	25
A. flavus	6.3	12.5	25	>25	25	>25
A. niger	12.5	>25	25	>25	25	>25
A. ochraceus	12.5	>25	12.5	>25	12.5	25
F. proliferatum	12.5	>25	25	>25	12.5	25

**Methicillin-resistant Staphylococcus aureus, MIC-minimum inhibitory concentration, MBC-minimum bactericidal concentration, MFC- minimum fungicidal concentration*

The best antimicrobial activity was observed in oak (*Quercus alba* L.) wood water with *K. pneumoniae*. It was observed that the oak wood water had no MBC activity with *P. vulgaris, B. cereus, S. typhimurium, A. niger, A. ochraceus, and F. proliferatum*, although MIC values were between 6.3 and 12.5 mg/mL (MBC > 25). The MBC values remained between 12.5 and 25 mg/mL despite MIC values were 6.3 to 12.5 mg/mL as for against other bacteria.

The MBC value of beech wood water with *P. aureginosa*, *S. Aureus*, *K. pneumoniae*, and *B. cereus* remained between 12.5 and 25 mg/mL despite MIC values between 6.3 to 12.5 mg/mL. The MBC activity was observed in beech (*Fagus orientalis* L.) wood water samples with *P. vulgaris, E. coli, MRSA, S. typhimurium, C. albicans, A. niger, A. ochraceus, A. flavus*, and *F. proliferatum*.

The MBC activity against six of the bacteria strains was observed in walnut (*Juglans regia* L.) wood water; however, the MBC values were observed between 12.5 and 25 mg/mL for all others.

Conclusion

According to the results of this study, minimal MIC values were determined in oak wood water obtained from oak (*Quercus alba* L.) log against *K. pneumonia* bacteria. The wood water from oak and walnut exhibited antimicrobial activity on seven species of bacteria, while beech wood water exhibited antimicrobial activity on four species of bacteria.

The HfV technical drying method was used in this study. The HfV method required only 1.5 to 9 days for drying the samples (Güler et al. 2012). And, this causes an increase in obtained drying water due to short cycling.

Beside this, there are lots of advantages of HfV method. These can be summarized as follows; heating source for drying process is electricity which does not release toxic gas to the environment, provides opportunity to dry thick logs, capability of uniform and selective drying (less humid regions consume less power while much humid regions consume much power), provides energy efficiency between 40% to 50%, low operational costs such as operating, maintenance and repair, etc., lower temperature requirement avoids defect formation such as cracking, warping, fiber breakage and etc. thanks to vacuuming (Dığrak et al. 1999), stowing the batch inside a vacuum kiln without wood lath/slat means close packing or efficient use of kiln volume and cost effectiveness too.

Results obtained from this study show that wood water samples have antibacterial activity. Therefore, results from this study imply the necessity for the evaluation of wood water as a reusable and value-added commodity for the plant-derived medicine industry in Turkey.

References

Abascal, K., Yarnell, E. (2002). Herbs and drug resistance: Potential of botanical in drug-resistant microbes. *J Altern. and Complement. Ther. 1* (pp.237-241).

Altınok, M., Küreli, İ., Serbes, T. (2009). The determination of the effect to some physical and mechanical properties of wood material of vacuum and conventional drying methods. *J Polytechnic 12(4)* (pp.271-278).

Alzoreky, N.S., Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int. J Food Microbiol. 80(3)* (pp.223-230).

Bouwmeester, J., Matusova, R., Zhongkui, S., Beale, M.H. (2003). Secondary metabolite signaling in host-parasitic plant interactions. *J Curr. Opin. in Plant Biol. 6(4)* (pp.358-364).

CLSI. (1997). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Ninth Edition*. Wayne, PA: Clin. and Lab. Stand. Inst.

Dığrak, M., İlçim, A., Alma, M.H. (1999). Antimicrobial activities of several parts of Pinus brutia, Juniperus oxycedrus, Abies cilicia, Cedrus libani and Pinus nigra. *J Phytotherapy Res. 13(7)* (pp.584-587).

Güler, C., Ünsal, Ö., Mollamehmetoğlu, G. (2012). High-Frequency-Vacuum wood drying technology. *J History Culture and Art Res. 1(*4) (pp.310-318).

İlçim, A., Dığrak, M., Bağcı, E. (1998). The investigation of antimicrobial effect of some plant extract. *Turkish J Biol. 22(1)* (pp.119-125).

Kalaycıoğlu, A., Öner, C. (1994). Antimutagenic effects investigation of some plant extracts by Amest-Salmonell test system (In Turkish: Bazı bitki ekstraktlarının antimutajenik etkilerinin Amest- Salmonella test sistemi ile araştırılması). *J Turkish Botany 18* (pp.117-122).

Korkut, S. (2011). New trend in lumber drying: High frequency (In Turkish: Kereste Kurutmada Yeni Trend: Yüksek Frekans). *Proceedings of the I. National Mediterranean Forest and Environment Symposium*, 26-28 October 2011, Kahramanmaraş, Turkey.

Resch, H. (2006). High-Frequency electric current for drying of wood-Historical perspectives. *J Maderas Cienc. y Tecnología 8(2)* (pp.67-82).

Resch, H., Hansmann, C. (2002). Tests to dry thick Eucalyptus boards in vacuum using high frequency heating. *Proceedings of the 4th COST E15 Workshop - Methods for Improving Drying Quality of Wood*, 30-31 May 2002, Santiago de Compostela, Spain.

Seyyednejad, S.M., Motamedi, H. (2010). A review on native medicinal plants in Khuzestan, Iran with antibacterial properties. *Intl. J Pharmacol. 6(5)* (pp.551-560).

Solanki, R. (2010). Some medicinal plants with antibacterial activity. *Pharmacie Globale: Int. J Compr. Pharm. 4(10)* (pp.1-4).

Tan, A. (1992). Plant diversity and plant genetic resources in Turkey (In Turkish: Türkiye'de Bitkisel Çeşitlilik ve Bitki Genetik Kaynakları). *Anadolu J AARI, MARA 2* (pp.50-64).

Toroğlu, S., Çenet, M. (2006). The methods used to determine antimicrobial activity of the plants and their application area. *Kahramanmaras Sutcu Imam Univ. J Sci. and Eng. 9(2)* (pp.12-20).

Tutuş, A., Kurt, R., Alma, M.H., Meriç, H. (2010). Chemical analysis of Scotch Pine wood and its thermal properties. *Proceedings of the III. National Black Sea Forestry Congress*, 20-22 May 2010, Artvin, Turkey.

Vonderbank, H. (1949). Ergebnisse der Chemotherapie der Tuberkulose (In English: Results of chemothreapy of tuberculosis), *J Pharm. 4* (pp.198-207).

Walsh, T.J., Anaissie, E.J., Denning, D.W., Herbrecht, R., Kontoyiannis, D.P., Marr, K.A., Morrison, V.A., Segal, B.H., Steinbach, W.J., Stevens, D.A., Burik, J.A., Wingard, J.R., Patterson, T.F. (2008). Treatment of aspergillosis: Clinical practice guidelines of the infectious diseases. *J Clin. Infect. Dis. 46(3)* (pp.327-360).

Zhao, J., Wang, J., Chen, Y., Agarwal, R. (1999). Anti-tumor promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two stage initiation promotion protocol and identification of procyanidin B5-3-gallate as the most effective antioxidant constituent. *J Carcinogenesis 20(9)* (pp.1737-1745).

