# Antimicrobial and Antifungal Properties of Madder Root (*Rubia tinctorum*) Colorant Used as an Environmentally-Friendly Wood Preservative

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The aim of this study was to determine the antifungal and antimicrobial properties of madder root extract when used as an environmentally-friendly wood preservative and against blue stain. Plant dyestuff was extracted from the root of madder by using an ultrasonic assisted method and then applied to Turkish oriental beech, Scots pine, oak, and walnut wood blocks with the immersion (classic) and immersion + ultrasonic assisted methods. For mordants, ferrous sulfate, aluminum sulfate, copper sulfate, and vinegar were used. In order to compare the performances of the natural paints, a synthetic dye was used. The abilities of the extracts to suppress attack by brown rot (*Postia placenta*) and white rot (*Trametes versicolor*) were investigated. Treated blocks were exposed to *P. placenta* and *T. versicolor* attacks for 16 weeks according to the TS 5563-EN 113 method. Antimicrobial activity of the extracts was determined with the agar dilution method by using the disk diffusion method for bacteria. Results showed that the mordant mixes were considerably more resistant to fungal decay compared to their untreated and synthetic counterparts. In general, control (non-mordant) and vinegar mixtures showed good performance against brown and white rot fungi. Copper mixes showed better antimicrobial activity against all types of microorganisms. In conclusion, it was found that madder root extracts and mordant mixes could be used as wood preservatives.

*Keywords: Madder root; Antifungal; Antimicrobial; White rot; Brown rot*

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## INTRODUCTION

Various chemicals and methods have been developed in the wood preservation industry for the protection and extended service life of wood material. In some cases, wood preservation chemicals developed for outdoor applications have been avoided for use in indoor applications due to their volatile organic compounds (VOC), which reduce air quality (Tascioglu *et al.* 2013).

Some problems have emerged in recent years, during the preservation and coloring of the wood material by chemicals, in terms of environmental health. In general, chemical substances that protect wood against harmful effects have a tendency to be toxic. For this reason, the possibility of protecting wood material by natural means is gaining importance. Increased awareness of health risks and overall indoor air quality has led to a demand for finishes and paints with lower VOC content.

Since ancient times natural dyes have been used in dyeing, and nowadays many natural dyes are regaining attention in various scientific studies. Goktas *et al.* (2010) investigated the antifungal properties of poisonous plant extracts from *Muscari neglectum* and *Gynandriris sisyrinchium*, and they reported that the extracts had an antifungal effect. In another study, Goktas *et al.* (2008) investigated the antifungal activities and color stability under ultraviolet (UV) exposure of wood treated with aqueous solutions of *Juglans regia* extracts, and they reported effective decay resistance of the extracts. Schultz and Nicholas (2002) reported that heartwood extracts might be alternative wood preservatives as they have fungicidal and antioxidant properties. More recently, Tung *et al.* (2007) reported that the ethanol extracts from the bark of *Acacia confusa* exhibited strong antioxidant activity. Yang and Clausen (2007) investigated antifungal effect of seven essential oils: derived-ajowan, dill weed, Egyptian geranium, lemongrass, rosemary, tea tree, and thyme, and their results indicated that these natural extracts had antibacterial and antifungal activity. Digrak *et al.* (2001) also reported antibacterial activity in extracts of mimosa bark. Tascioglu *et al.* (2013) investigated the antifungal properties of mimosa, quebracho, and pine bark extracts, and they reported that commercial mimosa and quebracho extracts can be utilized as alternative wood preservative chemicals against common wood decay fungi in door applications. Some other plant sources have rich naftakinons groups; for example lawsone in henna, juglone in walnut, and apachol in dyer's bugloss, have been found to have anti-bacterial and anti-fungal properties (Singh *et al*. 2005).

*Rubia tinctorium* is used as a diuretic and stone inhibitor and is endowed with genotoxic activity. Antifungal activities of *R. tinctorum* have been previously researched (Manojlovic *et al.* 2005). Turkey has a rich flora because of its geographical position and climate. It is well known that plant based dyes were widely used by the Turks in both central Asia and Anatolia throughout history (Calis *et al.* 2009). *Rubia tinctorium* is known for dyestuffs which originate from root material of field grown madder, including anthraquinone derivates, the most important of which is alizarin (The Merck 1996). Madder (*Rubia tinctorum*) contains several anthraquinone derivatives in its roots and rhizomes (Orbán *et al.* 2008). The main components are di- and trihydroxy-anthraquinones, alizarin, and purpurin and their derivatives (Gilbert and Cooke 2001). It is known that anthraquinone derivates have been used as anti-inflammatory, antimicrobic, antibacterial, and antidiuretic drugs (Swain 1996).

The focus of this study is on the antimicrobial effects and preservation of wood material against brown rot and white rot fungi by *Rubia tinctorum* extract, which is widely used as a dye in the carpet and textile industries. It is believed that finding eco-friendly colorants to overcome microorganism pollution will bring a new aspect with fewer side effects and less pollution.

## EXPERIMENTAL

### Materials

Specimens were prepared from wood that was free from cracks, stain, decay, insect damage, or other defects and had not been water-stored, floated, chemically treated, or steamed. Scots pine (*Pinus sylvestris* L.) sapwood, oriental beech (*Fagus orientalis* Lipsky), oak (*Quercus petraea* Liebl.), and walnut (*Juglans regia* L.) according to the TS 5563 EN 113, 1996 standards with a sample size of 50 x 25 x 15 mm (longitudinal x radial x tangential directions). All specimens were conditioned at 20 ±2 °C and 65 ± 3% RH for 3 weeks before the subsequent treatments.

Ferrous sulphate ($Fe_2(SO_4)_3.7H_2O$), aluminum sulphate ($Al_2(SO_4)_3.18H_2O$), and copper sulphate ($CuSO_2.5H_2O$) were provided from Kimetsan Co. Vinegar was purchased from Fersan Co.. The standard antibiotic discs used for comparison, such as Penicillin G, Ampicillin, Chloramphenicol, Gentamycin, Erythromycin, Nystatin, and Tetracyclin were purchased from OXOID Co. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538/P bacteria, and *Candida utilis* CCTM were used. Test microorganisms and fungal species were obtained from the culture collection of Mugla Sitki Kocman University, Faculty of Science, Mushroom Research Center Department.

## Methods

*Preparation of dyestuff*

The roots of *Rubia tinctorum* L. (Rubiaceae) were obtained from a herbalist located in Konya, Turkey. A weighed amount of dry plant material was extracted with distilled water in an ultrasonic bath (Elmasonic X-tra 150 H). Ultrasound-assisted extraction is an inexpensive, simple, and efficient alternative to conventional extraction techniques. The main benefits of using ultrasound in solid-liquid extraction include the increase of extraction yield and faster kinetics. Ultrasound can also reduce the operating temperature, allowing the extraction of thermolabile compounds. Compared with other novel extraction techniques such as microwave-assisted extraction, the ultrasound apparatus is cheaper and its operation is easier (Wang and Weller 2006). Power ultrasound can enhance a wide variety of chemical and physical processes, mainly due to the phenomenon known as cavitation in a liquid medium. Cavitation refers to the explosive collapse of microscopic bubbles after their growth. Sudden and explosive collapse of these bubbles can generate ‘‘hot spots’’ (Mason 1999, Suslick e*t al.* 1986), *i.e*. localised high temperature, high pressure shock waves and severe shear forces capable of breaking chemical bonds.

In the standard procedure the ratio of mass of plant material to the volume of liquid was 1:20. Extraction was performed for 180 min, at a temperature of 45 °C, and under 180 W of sonic power in a stainless ultrasonic bath. Due to the rather high liquor ratio, some manual stirring was sufficient to distribute the plant material in the liquid during the extraction period. Volume loss due to evaporation was compensated for by the addition of water at the end of the extraction period to obtain the initial volume.

Mordants were prepared by adding the following to aqueous solutions: 3% ferrous sulphate ($Fe_2(SO_4)_3.7H_2O$), 5% aluminum sulphate ($KAl_2(SO_4)_3.18H_2O$), 5% copper sulphate ($CuSO_2.5H_2O$), and 10% and grape vinegar. This was done in order to stabilize the color of dyes extracted, to ensure it would remain on the applied material (to increase retention amount), and to create color options.

*Treatment*

Wood specimens after oven-drying (maintained at 103 ± 2 °C) were cooled in desiccators to determine the initial dry mass, and then stored in order to keep them dry until impregnation. The air-dried wood specimens were placed into an ultrasonic bath container according to their intended treatments. Treatment procedures are given in Table 1. Two different methods (immersion and ultrasonic-assisted immersion) of dyeing were used. The weight percent gain (WPG) (%, w/w) due to chemical load was calculated from the following equation:

$$\text{WPG (\% w/w)} = \frac{W_{of} - W_{oi}}{W_{oi}} \times 100 \quad (1)$$

In this equation, $W_{oi}$ is the oven-dry weight (g) of a wood specimen before impregnation and $W_{of}$ is the oven-dry weight (g) of a wood specimen after impregnation. The treated wood blocks were stored for four weeks according to TS 5563 EN 113 in a conditioning room at 20 ± 2 °C and 65 ± 3% relative humidity until they reached a stable weight before the decay resistance tests.

**Table 1.** Treatment Procedures

| Dye | Treatment Method | Sonic Power (W) | Temperature (°C) | Time (min) |
|---|---|---|---|---|
| Natural | Control (immersion) | --- | 45 | 60 |
| Synthetic* | Ultrasonic-assisted immersion | 300 | 45 | 60 |

(*) Genç Fulltex Wood Colourant Properties: Cellulosic alkyd based thinning with the use of thinner, Solid content (%) 5 to 25, Density (kg/lt ) 0.79 to 0.85

*Decay resistance test*

Decay resistance and antimicrobial tests were conducted in Mugla Sitki Kocman University, Faculty of Science, Mushroom Research Center Laboratory. Untreated and treated wood specimens were exposed to four different Basidiomycetes fungi according to the EN 113 (TS 5563) standard. In this experiment a white rot fungi, *Trametes versicolor* (L: Fr.) Pilat. (FFPRI 1030) and a brown rot fungi *Postia placenta* (Fr.) M.J. Larsen & Lombard (Mad-698-R) were used. *Postia placenta* was maintained on 0.39% potato dextrose agar (PDA) medium, while *Trametes versicolor* was grown on 0.48% malt extract agar (MEA) medium. The media was then steam sterilized at 120 ± 2 °C for 15 min before being transferred to pre-sterilized petri dishes. After inoculation, the dishes were kept at 26 ± 2 °C and 70 ± 2% relative humidity until the media surfaces were completely colonized by the test fungi. The treated and untreated wood blocks were sterilized at 120 °C for 15 min after their oven-dried reference weights were determined. Five specimens per group were placed in pre-inoculated petri dishes on solid maple feeder strips to minimize direct contact with nutritional media surfaces. Following fungal exposure for 16 weeks at 26 ± 2 °C and 70 ± 2% relative humidity in an incubator, the exposed wood specimens were weighed immediately after the surface mycelium was cleaned. Percent mass losses were calculated from the difference according to TS 5563 EN 113 in the 103± 2 °C oven-dried weights of each specimen before and after the decay test.

$$\text{Mass loss (\%)} = [100 \times (T_1 - T_2) / T_1)] \quad (2)$$

In this equation, $T_1$ is the weight of the wood specimen plus remaining preservative after conditioning and before exposure to the test fungus, and $T_2$ is the weight of the wood specimen after test and after final conditioning.

*Disc-diffusion assay*

Extracts were sterilized by filtration with a 0.45 μm Millipore filters for antimicrobial tests. Antimicrobial tests were then carried out by the disc-diffusion method (Murray *et al.* 1995) using 100 μL of suspension containing 108 CFU/mL of bacteria, 106 CFU/mL of yeast, and 104 spore/mL of fungi spread on nutrient agar (NA), sabour and dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. Empty sterilized discs of 6 mm were each impregnated with 50 μL madder root and its mordant mixes. The inoculated plates were incubated at 28 °C for 12 h for clinical bacterial strains and 48 h for yeast isolates. Plant-associated microorganisms were incubated at 28 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms at 24 and 48 h. Each assay in this experiment was repeated twice.

## RESULTS AND DISCUSSION

Mean extract retentions, calculated based on gross solution uptake data and concentration of treated wood specimens, are given in Table 2. When wood species treated by natural extract were compared, the highest retentions were recorded for Scots pine sapwood 5.73% on control group treated with the classic method, and the lowest retentions were recorded for walnut 1.59% on madder root and alum mixture. Heterogeneous texture, density, and porosity values of wood species used played an important role on retention values (Aydin and Colakoglu 2003). On the other hand, particle size of extracts is responsible for penetration efficiency of the retention rate.

**Table 2.** Mean Extract Retentions (%) of Wood Species Treated With Different Extracts

| | | Retention (%) | | | |
|---|---|---|---|---|---|
| Extracts | Treatment method | Scots pine | Beech | Walnut | Oak |
| Control (Non mordant) | Ultrasonic | 4.13 (0.78) | 3.10 (0.88) | 3.02 (0.12) | 4.94 (0.45) |
| | Classic | 5.73 (0.56) | 4.04 (0.23) | 2.70 (0.56) | 3.37 (0.47) |
| Root + Ferrous | Ultrasonic | 4.66 (0.71) | 3.88 (0.47) | 2.69 (0.55) | 3.95 (0.45) |
| | Classic | 4.20 (0.31) | 3.83 (0.61) | 2.59 (0.23) | 3.63 (0.12) |
| Root + Alum | Ultrasonic | 4.75 (0.25) | 4.61 (0.26) | 1.59 (0.59) | 4.81 (0.84) |
| | Classic | 3.46 (0.36) | 3.47 (0.36) | 2.78 (0.45) | 3.37 (0.91) |
| Root + Copper | Ultrasonic | 5.66 (0.44) | 3.83 (0.22) | 2.25 (0.66) | 4.77 (0.88) |
| | Classic | 4.30 (0.27) | 4.24 (0.58) | 2.44 (0.32) | 4.39 (0.74) |
| Root + Vinegar | Ultrasonic | 3.35 (0.33) | 3.75 (0.36) | 2.25 (0.41) | 3.85 (0.61) |
| | Classic | 3.06 (0.18) | 3.55 (0.66) | 2.11 (0.45) | 3.40 (0.64) |
| Synthetic | Ultrasonic | 3.59 (0.18) | 5.85 (0.83) | 2.73 (0.36) | 1.84 (0.29) |
| | Classic | 4.61 (0.22) | 5.78 (0.44) | 2.95 (0.27) | 2.17 (0.72) |

Mean of 5 replicates, numbers in parenthesis are standard deviations

**Table 3.** Multiple Variance Analysis for Retention Rate

| Factors | Sum of squares | Degrees of freedom | Mean square | *F*-value | *P*-value* |
|---|---|---|---|---|---|
| A: Treatment method | 1.129 | 2 | .564 | 11.464 | .000 |
| B: Wood species | 106.824 | 3 | 35.608 | 723.453 | .000 |
| C: Mordant agent | 14.489 | 5 | 2.898 | 58.876 | .000 |
| Interaction A*B | 4.328 | 6 | .721 | 14.657 | .000 |
| Interaction A*C | 5.464 | 10 | .546 | 11.102 | .000 |
| Interaction B*C | 76.708 | 15 | 5.114 | 103.900 | .000 |
| Interaction A*B*C | 22.154 | 30 | .738 | 15.004 | .000 |
| Error | 8.269 | 168 | .049 | | |
| Total | 3488.769 | 240 | | | |

The effects of all factors on retention rate data were found to be statistically significant (Table 3).

## Mass Losses

*Brown rot fungi*

Mass losses of the wood species treated with extract solutions and exposed to *Postia placenta* brown rot fungus for 16 weeks are given in Table 4.

**Table 4.** Mass Losses of Wood Species Treated With Madder Root and Mordant Mixes After 16 Week Exposure to *Postia placenta*

| Extracts | TM | Weight Loss (%) | | | |
|---|---|---|---|---|---|
| | | Wood Species | | | |
| | | Scots pine | Beech | Walnut | Oak |
| | | X (SD) | X (SD) | X (SD) | X (SD) |
| Untreated | --- | 26.17 (7.68) *abc* | 29.37 (2.26) *e* | 18.25 (0.82) *c* | 9.26 (0.61) *bc* |
| Control (Non mordant) | U | 24.88 (4.55) *abc* | 24.97 (2.96) *cde* | 13.95 (5.36) *abc* | 8.49 (1.63) *bc* |
| | C | 25.26 (3.64) *abc* | 25.81 (4.08) *de* | 13.40 (3.50) *abc* | 8.09 (0.84) *bc* |
| Root + Ferrous | U | 22.89 (3.26) *ab* | 19.46 (0.96) *b* | 16.18 (3.04) *c* | 8.34 (0.82) *bc* |
| | C | 27.25 (3.00) *bc* | 27.25 (6.06) *de* | 12.58 (4.42) *abc* | 8.15 (0.18) *bc* |
| Root + Alum | U | 19.95 (1.88) *a* | 18.28 (2.17) *b* | 16.76 (5.33) *c* | 8.55 (1.50) *bc* |
| | C | 22.89 (1.72) *ab* | 22.75 (6.74) b*cd* | 15.65 (4.61) *c* | 7.93 (0.52) *bc* |
| Root + Copper | U | 22.73 (4.15) *ab* | 22.75 (6.74) *cde* | 13.83 (3.69) *abc* | 9.77 (2.57) *bc* |
| | C | 18.25 (5.82) *a* | 14.01 (6.60) *a* | 9.80 (2.86) *ab* | 9.89 (3.84) *c* |
| Root + Vinegar | U | 30.00 (5.64) *c* | 27.69 (5.03) *de* | 13.25 (2.58) *abc* | 8.21 (1.42) *ab* |
| | C | 26.62 (4.68) *bc* | 19.96 (4.68) *bc* | 8.31 (1.32) *a* | 7.80 (0.43) *b* |
| Synthetic | U | 27.19 (3.04) *bc* | 13.30 (1.37) *a* | 14.64 (4.23) *bc* | 5.42 (0.59) *a* |
| | C | 26.09 (4.71) *bc* | 10.48 (2.89) *a* | 9.19 (4.30) *abc* | 6.11 (0.59) *a* |

TM: Treatment method, U: Ultrasonic, C: Classic; a,b,c,d,e : Presented homogeneity groups.

Untreated control specimens of Scots pine, oriental beech, walnut, and oak wood were severely attacked by decay caused by *Postia placenta* fungi, resulting in large weight losses. According to the results, mass losses of madder root (non-mordant) treated wood species showed weak resistance to the brown rot fungus compared to the untreated specimen. Wood species impregnated with madder root and mordant mixtures (except Scots pine impregnated by vinegar and oak impregnated by copper) revealed lower mass losses during exposure to *Postia placenta* compared with untreated groups. On the other hand, Scots pine species' mass losses for madder root and mordant mixtures (except vinegar) showed better resistance against brown rot fungi when compared to synthetic specimens.

The effects of wood species, mordant agent, interaction of treatment method and mordant agent, interaction of wood species and mordant agent, and interaction of treatment method, wood species, and mordant agent on mass loss data were evaluated for brown rot fungi and found to be statistically significant (Table 5).

**Table 5.** Multiple Variance Analysis for Mass Losses of *Postia placenta*

| Factors | Degrees of freedom | Mean square | *F*-value | *P*-value* |
|---|---|---|---|---|
| A: Treatment method | 1 | 46.47 | 3.13 | .079 NS |
| B: Wood species | 3 | 2718.02 | 183.08 | .000* |
| C: Mordant agent | 4 | 59.84 | 4.03 | .004* |
| Interaction A*B | 3 | 20.51 | 1.38 | .250 NS |
| Interaction A*C | 4 | 92.53 | 6.23 | .000* |
| Interaction B*C | 12 | 56.59 | 3.81 | .000* |
| Interaction A*B*C | 12 | 29.46 | 1.98 | .029* |
| Error | 160 | 14.85 | | |
| Total | 200 | | | |

NS : Not Significant

*White rot fungi*

Mass losses of the wood species treated with extract solutions and exposed to *Trametes versicolor* white rot fungus for 16 weeks are given in Table 6. According to the results, madder root (non-mordant) impregnation on Scots pine and oriental beech increased mass losses, whereas mass losses were decreased on the walnut and oak species. Generally, mordant agents decreased the fungal activities compared to untreated control specimens.

Scots pine impregnated by ferrous and alum mixes, oriental beech impregnated by ferrous, alum, and copper mixes, walnut impregnated by ferrous, copper, and vinegar mixes, and oak species impregnated by ferrous and vinegar mixes showed lower mass losses when compared with impregnated synthetic specimens. Control (non-mordant) impregnation on Scots pine and oriental beech specimens resulted in increased mass losses. Walnut and oak specimens impregnated with control (non-mordant) and vinegar mixtures showed preservation.

**Table 6.** Mass Losses of Wood Species Treated With Madder Root and Mordant Mixes After 16 Week Exposure to *Trametes Versicolor*

| Extracts | TM | Weight Loss (%) | | | |
|---|---|---|---|---|---|
| | | Wood Species | | | |
| | | Scots Pine | Beech | Walnut | Oak |
| | | X (SD) | X (SD) | X (SD) | X (SD) |
| Untreated | --- | 6.17 (1.87) *cde* | 11.75 (3.91) *a* | 22.69 (2.97) *e* | 6.77 (0.95) *abcd* |
| Control (Non mordant) | U | 12.09 (1.49) *f* | 25.34 (2.70) *cd* | 7.96 (4.41) *bcd* | 6.97 (3.56) *bcd* |
| | C | 13.69 (3.96) *f* | 25.82 (6.17) *cd* | 10.22 (3.86) *d* | 8.29 (3.27) *cd* |
| Root + Ferrous | U | 3.56 (0.70) *a* | 8.24 (2.48) *a* | 6.64 (2.10) *abc* | 3.82 (1.28) *a* |
| | C | 3.67 (0.48) *ab* | 9.93 (1.91) *a* | 4.43 (1.18) *a* | 6.02 (4.12) *abc* |
| Root + Alum | U | 4.52 (1.23) *abc* | 12.67 (3.87) *ab* | 8.47 (1.32) *cd* | 6.78 (2.36) *abcd* |
| | C | 5.13 (0.80) *abcd* | 11.69 (3.21) *a* | 9.61 (0.78) *cd* | 9.59 (1.30) *d* |
| Root + Copper | U | 7.75 (0.98) *e* | 8.24 (0.88) *a* | 6.82 (0.56) *abc* | 8.63 (0.96) *cd* |
| | C | 7.35 (1.22) *e* | 9.29 (1.27) *a* | 7.08 (0.59) *abcd* | 6.77 (2.00) *abcd* |
| Root + Vinegar | U | 6.90 (1.12) *de* | 28.70 (7.75) *d* | 9.01 (1.08) *cd* | 3.94 (1.15) *a* |
| | C | 4.80 (0.49) *abc* | 24.05 (1.56) *c* | 4.96 (2.33) *a* | 5.03 (2.87) *ab* |
| Synthetic | U | 5.16 (0.77) *abcd* | 16.50 (1.88) *b* | 8.28 (3.99) *cd* | 6.45 (2.26) *abc* |
| | C | 5.46 (0.77) *bcd* | 16.05 (2.42) *b* | 9.35 (4.10) *cd* | 6.54 (1.93) *abc* |

TM: Treatment method, U: Ultrasonic, C: Classic

The effects of wood species, mordant agent, interaction of treatment method and mordant agent, and interaction of wood species and mordant agent on mass loss data were evaluated for brown rot fungi and found to be statistically significant, as shown in Table 7.

**Table 7.** Multiple Variance Analysis for Mass Losses of *Trametes versicolor*

| Factors | Degrees of Freedom | Mean Square | *F*-value | *P*-value* |
|---|---|---|---|---|
| A: Treatment method | 1 | .02 | .00 | .957$^{NS}$ |
| B: Wood species | 3 | 1107.39 | 156.14 | .000* |
| C: Mordant agent | 4 | 381.49 | 53.79 | .000* |
| Interaction A*B | 3 | 7.27 | 1.03 | .383$^{NS}$ |
| Interaction A*C | 4 | 22.34 | 3.150 | .016 $^{NS}$ |
| Interaction B*C | 12 | 195.53 | 27.57 | .000* |
| Interaction A*B*C | 12 | 7.65 | 1.08 | .382$^{NS}$ |
| Error | 160 | 7.09 | | |
| Total | 200 | | | |

NS: Not Significant

In general, the mass losses showed variable responses against brown and white rot fungus. It is well known that the natural durability changes among wood species as well as within the same species depending on tree age, growth region, conditions, and seasonal differences (Bozkurt *et al.* 1993). In some cases the mordant agent mix treated wood specimens (beech, walnut, and oak) exhibited slightly higher mass losses when compared with impregnated synthetic specimens and untreated specimens. The mordant agents have the ability to make a complex chemical bond with the wood components (which has -OH groups). This may have effects on the fungal attack. On the other hand, structural changes among wood species depend on the complex structure and different reagent groups (carbonyl, ether, acid, hydroxyl groups). These changes affect the fixation of chemical materials to the wood (Vasishth 1996). Generally, control (non-mordant) and vinegar mixtures showed good performance against brown and white rot fungi. Furthermore, dyeing method did not affect the mass losses against all types of fungus.

## Antimicrobial Activity

Table 8 shows antibacterial activities of the madder root and mordant mixes. These mixes were screened for antimicrobial activities against selected bacteria (*E. coli* and *S. aureus*) and fungus (*C. utilis*). In addition, the inhibition zones and for comparison standard antibiotic discs are indicated in Table 8.

**Table 8.** Antimicrobial Activity of Madder Root-Mordant Mixes and Some Standard Antibiotics (Inhibition Zone, mm*)

| | | Microorganism | | |
|---|---|---|---|---|
| | | *Escherichia coli* | *Staphylococcus aureus* | *Candida utilis* |
| Extracts | Control (Non mordant) | - | - | - |
| | Root + Ferrous | - | 8 | - |
| | Root + Alum | 8 | - | - |
| | Root + Copper | 9 | 21 | 8 |
| | Root + Vinegar | - | - | - |
| | Synthetic | - | - | - |
| Antibiotics | Chloramphenicol | 21 | 29 | - |
| | Gentamycin | 25 | 38 | - |
| | Erythromycin | 13 | 14 | - |
| | Nystatin | - | - | 27 |
| | Penicillin | - | - | - |
| | Tetracycline | 21 | 26 | - |
| | Ampicillin | 14 | 9 | - |

Inactive (-); moderately active (7-13); highly active (> 14)
*Includes diameter of disk (6 mm)

According to the results, the extracts of madder root mixed with copper had the highest antibacterial efficiency on all microorganisms. The madder root mixed with ferrous sulfate having inhibition zone on *Staphylococcus aureus* and madder root mixes with alum was determined to have antimicrobial effects on *Escherichia coli*, while the others (control, root+vinegar, and synthetic) did not (Table 8).

When the results obtained from madder root and mordant mixes were compared with standard antibiotics (Table 8), it was determined that *S. aureus* was mildly resistant, while *E. coli* and *C. utilis* were susceptible to madder root and copper mixes. Ferrous mixed of madder root against *S. aureus*, alum and copper mixed against *E. coli* showed lesser resistance compared to standard antibiotics (except nystatin and penicillin). On the other hand, copper mixed of madder root exhibited more resistance against *S. aureus* than Erythromycin and Ampicillin.

According to the literature, the water base of *R. tinctorum* did not show antibacterial activity against *E. coli* and *S. aureus* (Kalyoncu 2006). In this study, the results for the control (non-mordant) were similar to those in the cited study. Otherwise the root extract and copper mixes showed antimicrobial activity against *E. coli*, *S. aureus* and *C. utilis* 9 mm, 21 mm, and 8 mm, respectively. Recent studies indicate that bacteria, including certain harmful strains of *E. coli* and *S. aureus*, can cause serious nosocomial or hospital acquired infections, but will simply die in a few hours when placed on copper alloy surfaces at room temperature (Lewis 2005).

## CONCLUSION

1. The range of the mass loss of wood specimens treated with mordant mixes showed considerable resistance to fungal decay compared to the untreated and synthetic specimens. In some cases, control (non-mordant) and vinegar mixtures showed good performance against brown and white rot fungi.
2. Madder root and copper mixes showed better antimicrobial activity against all types of microorganisms compared with other extracts and synthetic dyes.
3. The intention of the development of more environmentally friendly wood treatments should encourage scientists to open the door to use plant extracts as wood preservatives and colorants.
4. The plant extracts can offer substantial advantages for wood protection and aesthetics at low cost, low mammalian toxicity, and ease of handling and treatment. Moreover, for the assessment of plants that are naturally grown in Turkey and are potential rich sources, studies will be very efficacious from economic and ecological standpoints.

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