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# DETERMINATION OF ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF NATURAL DYE OF POMEGRANATE SKIN (*PUNICA GRANATUM* L.) IMPLEMENTED ON WOODEN MATERIALS

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## ABSTRACT

The demand for partially harmless dyes produced from natural sources as an alternative to synthetic dyes has been increasing. Natural dyes environment and they are attractive materials contributing to the protection of natural balance and to the reduction of aesthetic concerns of people. Yet, for natural dyes to be long-lasting and cling to the surface, they need to be used with mordant substances.

In the present study, the purpose is to determine the antifungal and antibacterial activities of natural dye obtained from pomegranate skin by means of ultrasonic. Plant extract obtained through ultrasonic method was applied to oriental beech (*Fagus orientalis* L.) and yellow pine (*Pinus sylvestris* L.) wooden materials by means of dipping and ultrasonic-assisted immersion. For the analysis of the results, data obtained from the natural dye were compared with those obtained from the synthetic dye. The findings of the analysis revealed that the most effective results in terms of antifungal activities were obtained from the solutions applied to yellow pine wood. For beech samples, the natural dye yielded better results when compared to the synthetic dye. Moreover, pomegranate skin solutions were found to prevent the spread of antibacterial activities. As a result, it was concluded that pomegranate skin extract can be used as wood preservative and coloring materials by mixing with holding provider.

KEYWORDS: Natural wood stain, mordants, ultrasonic assisted dyeing, *Trametes versicolor*, *Rhodonia placenta*, antimicrobial, pomegranate skin.

## INTRODUCTION

Attempts have been made in develop harmless substances as alternative to all materials harmful to human health and environment. With increasing importance attached to human health and environment, states have been taking new protective measures and accordingly, natural dyes have been favored against synthetic and harmful dyes by people (Kamel et al. 2005), (Calogero and Marco 2008), (Tsatsaroni et al. 1998).

Dye plants have been mostly discovered by Eastern societies throughout the history. Attractive colorful clothes were produced in China, India and Iran and traded to the West through Silk Road. The most valuable dye plants grown in these regions were saffron giving yellow color, dyer's woad yielding blue color and madder providing red color (Durmuskahya 2006)

Though natural dyes have been used for coloring for centuries, there is a lack of scientific research from many aspects. In recent years, importance attached to natural dyes as environmental friendly materials has increased. Although synthetic dyes are cost-effective, natural dyes can compete with them due to variety of their sources (Dixit and Jahan 2005).

Turkey is one of the leading countries in pomegranate production and there are many wastes such as pomegranate seed and skin resulting from pomegranate juice production. Due to its antioxidant content, the fruit has the potential to prevent cancer and cardiovascular diseases; yet, there is not enough research conducted on the seeds and skin of the fruit (Sagdic et al. 2011).

As the pomegranate plant is naturally a small tree, it has some advantages such as planting a lot of trees in relatively small areas, and accordingly getting high yield from small areas, easy harvest, durability during storage and transportation and long marketing period. Though it can be grown in almost every region of Turkey, it is mostly grown in Aegean, Mediterranean and South Anatolia regions of the country (Ogutgen 2008).

Pomegranate skin is rich in tannin, it is widely used in leather processing industry, in the clarification of fruit juices and for the prevention of zinc poisoning. Moreover, pomegranate skin and flower are used in the production of dye and ink. Pomegranate seeds include as much oil as cotton seeds. Remaining pulp of it in oil production industry is the richest herbal source of estrogen hormone (Anonymous 2014).

Ancient people had only extracts of plants to color objects. However, some of these extracts provided dyes resistant to washing and light, some others were not useful coloring agents and as a result people started to seek for different alternatives. In 2000 B.C., probably in India, after the invention of mordanting, it became possible to use many coloring agents not useful until then (Karadag 2007).

Through the mixture of mordants with coloring agents, the coloring agents are allowed to holding fibers better and different color combinations of a coloring agent can be obtained. As mordant materials, different substances ranging from metal salts that are dissolved in water to substances exhibiting weak acidic and basic characteristics can be used. Though there are many metal salts that can be used in natural dying and iron salts that are not toxic, carcinogenic and not causing environmental pollution have been used. Though the other metal salts have wider color spectra, they are not preferred as they are toxic, carcinogenic and cause environmental pollution (Karadag 2007).

Within the context of the current study, the aim was to determine the protectiveness of extracts derived from pomegranate skin on wooden materials and its antibacterial activities.

# MATERIAL AND METHODS

## Wooden material

The pomegranate skin used in this study were collected from the region of Muğla-Turkey. The collected samples were air dried and kept in the Herbarium of Mugla Sitki Kocman University-Turkey.

For the impregnation process, wood specimens were prepared from Scots pine (*Pinus sylvestris* L.) sapwood and beech (*Fagus orientalis* L.) according to the TS 5563 EN 113, 1996 standards with a sample size of 15 (radial) x 25 (tangential) x 50 mm (longitudinal). All specimens stored in the laboratory conditions at 20 ±2°C and 65 ± 5 % relative humidity of air for 3 weeks before the subsequent treatments in order to reach equilibrium content.

## Methods

### Preparation of dyestuff

A weighed amount of dry plant material was extracted with distilled water in an ultrasonic bath (Elmasonic X-tra 150 H). In the standard procedure the ratio of mass of plant material to the volume of liquid was 50 % (Ozen et al. 2014a). Extraction was performed for time of 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: 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 (Ozen et al. 2014b).

### Treatment

Air-dried wood specimens were placed into an ultrasonic bath container according to their intended treatments. Treatment procedures are given in Tab. 1. In the treatment two different methods (immersion and ultrasonic-assisted immersion) of dyeing were used.

*Tab. 1: Parameters of treatment procedures.*

| Dyes | Treatment method | Sonic Power (W) | Temperature (°C) | Time (min) |
|---|---|---|---|---|
| Natural dye | Immersion | --- | 45 | 60 |
| | Ultrasonic-assisted immersion | 300 | 45 | 60 |
| Synthetic wood dye | Immersion | --- | 45 | 60 |
| | Ultrasonic-assisted immersion | 300 | 45 | 60 |

The weight percent gain (WPG) (%, w/w) due to chemical load was calculated from the following equation:

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

where: $W_{oi}$ - oven-dry weight (g) of a wood specimen before impregnation,
$W_{of}$ - oven-dry weight (g) of a wood specimen after impregnation.

### Decay resistance test

The treated wood blocks were stored in a conditioning room at 20 ± 2°C and 65 ± 3 % relative humidity according to standard TS 5563 EN 113 (1996) until they reached a stable weight before the decay resistance tests.

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 two different Basidomycetes fungi according to EN 113 (TS 5563) (1996) standard. In this experiment a white rot fungi, *Trametes versicolor* (L: Fr.) Pilat. (FFPRI 1030) and a brown rot fungi *Rhodonia placenta* (Fr.) M.J. Larsen & Lombard (Mad-698-R) were used. *Rhodonia placenta* was maintained on 0.39 % potato dextrose agar (PDA) medium, while *T. 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 in the 60°C oven-dried weights of each specimen before and after the decay test.

*Disc-diffusion assay*

Extracts were sterilized by filtration with a 0.45 μm millipore filter for antimicrobial tests.

The standard antibiotic discs used for comparison, such as Penicillin G, Ampicillin, Chloramphenicol, Gentamycin, Erythromycin, Nystatin, and Tetracyclin were purchased from OXOID Co. *Staphylococcus aureus* ATCC 6538/P bacteria, *Candida utilis* CCTM, and *Bacillus megaterium* 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.

Antimicrobial tests were then carried out by 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), sabourand dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The discs (6 mm in diameter) were impregnated with 5 μL of the extracts and placed on the inoculated agar. The inoculated plates were incubated at 28°C for 12 hours for clinical bacterial strains and 48 hours 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 hours in milimeters (mm). Each assay in this experiment was repeated twice.

## RESULTS AND DISCUSSION

Means extracts retentions, calculated based on total solution uptake of treated wood specimens are given in Tab. 2. The highest retentions were recorded for Scotch pine 7.31 % on pomegranate skin + aluminium group treated with the ultrasonic method, and the lowest retentions were recorded for beach 4.62 % on pomegranate skin + vinegar mixture treated with ultrasonic application.

*Tab. 2: Mean extract retentions of wood species treated with different extracts.*

| Weight percent gain (WPG) (%, w/w) | | | |
|---|---|---|---|
| **Extracts** | **Treatment method** | **Beech X (SD)** | **Scotch pine X (SD)** |
| Control (Non mordant) | Ultrasonic | 4.84 (0.72) | 5.70 (0.55) |
| | Classic | 5.31 (0.89) | 5.33 (0.03) |
| Pomegranate skin + aluminium | Ultrasonic-assisted | 4.90 (0.12) | 7.31 (1.13) |
| | immersion | 5.10 (0.12) | 6.62 (1.01) |
| Pomegranate skin + copper | Ultrasonic-assisted | 5.23 (0.12) | 5.78 (0.46) |
| | immersion | 5.58 (0.58) | 5.60 (0.17) |
| Pomegranate skin + vinegar | Ultrasonic-assisted | 4.62 (0.06) | 5.62 (0.06) |
| | immersion | 5.44 (1.43) | 5.32 (0.59) |

Mass losses of the wood species treated with extract solutions and exposed to *Trametes versicolor* white rot fungus for 16 weeks are given in Tab. 3. Scotch pine impregnated by aluminium, copper and vinegar mixes were showed higher mass losses when compared with impregnated synthetic specimens.

*Tab. 3: Mass losses - Δm (%) of wood species treated with pomegranate skin and mordant mixes after 16 week exposure to fungus Trametes versicolor.*

| Mass losses- Δm (%) | | | |
|---|---|---|---|
| **Extracts** | **Treatment method** | **Beech X (SD.)** | **Scotch pine X (SD.)** |
| Control (Non mordant) | Ultrasonic | 17.31 (0.65) | 7.63 (1.06) |
| | Classic | 17.28 (0.67) | 7.76 (0.60) |
| Pomegranate skin + aluminium | Ultrasonic | 22.57 (2.42) | 5.81 (1.22) |
| | Classic | 23.16 (1.21) | 6.08 (1.71) |
| Pomegranate skin + copper | Ultrasonic | 23.37 (1.83) | 11.35 (0.65) |
| | Classic | 23.45 (0.34) | 11.91 (0.99) |
| Pomegranate skin + vinegar | Ultrasonic | 23.50 (1.48) | 9.01 (0.88) |
| | Classic | 23.79 (1.12) | 9.43 (0.92) |
| Synthetic wood dye | Ultrasonic | 18.3 (1.42) | 9.7 (1.67) |
| | Classic | 19.8 (1.15) | 21.7 (1.94) |

* Numbers in parenthesis are standard deviations (SD).

Mass losses of the wood species treated with extract solutions and exposed to *Rhodonia placenta* brown rot fungus for 16 weeks are given in Tab. 4. According to the results, mass losses of pomegranate skin (non-mordant) treated wood species showed low resistance to the brown rot fungus compared to the treated specimens. Wood species impregnated with pomegranate skin and mordant mixtures revealed high mass losses during exposure to *Rhodonia placenta* compared with untreated groups. The effects of wood species, mordant agent, interaction of wood specimens and mordant agent on mass loss data were evaluated for brown rot fungi and found to be statistically significant (Tab. 5).

*Tab. 4: Mass losses of wood species treated with pomegranate skin and mordant mixes after 16 week exposure to Rhodonia placenta.*

| Mass losses (%) – modify as Tab. 3 | | | |
|---|---|---|---|
| **Extracts** | **Treatment method** | **Beech X (SD.)** | **Scotch pine X (SD.)** |
| Control (Non mordant) | Ultrasonic | 15.44 (0.99) | 10.12 (1.14) |
| | Classic | 15.59 (1.47) | 10.45 (0.94) |
| Pomegranate skin + aluminium | Ultrasonic | 15.8 (1.81) | 11.95 (0.81) |
| | Classic | 15.73 (1.47) | 12.17(0.71) |
| Pomegranate skin + copper | Ultrasonic | 21.52 (1.86) | 13.74 (0.50) |
| | Classic | 21.67 (1.06) | 13.79 (0.39) |
| Pomegranate skin + vinegar | Ultrasonic | 22.38 (1.38) | 6.46 (0.74) |
| | Classic | 22.95 (0.85) | 7.89 (1.02) |
| Synthetic wood dye | Ultrasonic | 23.4 (1.4) | 27.2 (3.1) |
| | Classic | 24.9 (2.5) | 29.1 (2.5) |

*Tab. 5: Multiple variance analysis for mass losses.*

| **Factors** | **Sum of squares** | **Mean square** | ***F*-value** | ***P*-value*** |
|---|---|---|---|---|
| A: Wood species | 1 | 4356.26 | 607.75 | 0.00 |
| B: Mordant agent | 3 | 325.42 | 45.40 | 0.00 |
| C: Treatment method | 1 | 18.56 | 2.59 | 0.112 |
| Interaction A*B | 3 | 252.07 | 35.16 | 0.00 |
| Interaction A*C | 1 | 6.64 | 0.92 | 0.339 |
| Interaction B*C | 3 | 8.47 | 1.18 | 0.324 |
| Interaction A*B*C | 3 | 2.31 | 0.32 | 0.809 |
| Error | 64 | 7.16 | | |
| Total | 80 | | | |

Among the treatment samples on which pomegranate skin coloring agent was administered, the most effective results against white decay (*Trametes versicolor*) fungus were obtained for scotch pine tree with aluminum sulphate administered through ultrasonic method (yellow pine tree, 5.81 %) and for beech tree, the most effective results were obtained from the control samples (beech tree, 17.28 %). The most effective results against brown decay (*Rhodonia placenta*) fungus were obtained from the control samples of beech tree treated with ultrasonic method (15.44 %) and for yellow pine tree, the most effective results were obtained from vinegar mordant implemented with ultrasonic method (6.46 %). Against brown decay fungus, all the results obtained for all the treatment samples are better than those obtained through synthetic dye.

In general, the results showed that 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; Ozen et al. 2014b). The mordant agents have the ability to make a complex chemical bond with the wood components (which has -OH groups). 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; Ozen et al. 2014b).

## Antimicrobial activity

It is also clear from Tab. 6 that antibacterial activities of the pomegranate skin extract and mordant mixes was screened for antimicrobial activities against selected bacteria (*Staphylococcus aureus* and *Bacillus megaterium*) and fungus (*Candida albicans*).

*Tab. 6: Antimicrobial activity of pomegranate skin dye and mordant mixes.*

| Extracts | *Inhibition zone (mm*)* | | |
|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Bacillus megaterium*** | ***Candida albicans*** |
| Control (Non mordant) | 13 | 0.7 | 0 |
| Pomegranate skin + aluminium | 0.9 | 0.8 | 0 |
| Pomegranate skin + copper | 14 | 16 | 15 |
| Pomegranate skin + vinegar | 14 | 0 | 0 |
| Synthetic wood dye | 0 | 0 | 0 |

Inactive (-); moderately active (7-13); highly active (> 14)

*Includes diameter of disk (6 mm).

According to the results, the extracts of pomegranate skin and all mordant mixes were showed antimicrobial effectiveness against *Staphylococcus aureus* and *Bacillus megaterium* microorganism (expect pomegranate skin+vinegar) while synthetic did not. Otherwise the pomegranate skin extracts and copper mixes showed more efficient antimicrobial activity against *Staphylococcus aureus*, *Bacillus megaterium* and *Candida albicans* 14, 16, and 15 mm respectively.

According to the literature, the copper mixes of Pomegranate skin extract showed the best antibacterial activity against *E. coli, S. aureus* and *C.utilis* (Ozen et al. 2014b). In this study, the results for the copper mixes were similar to those in the cited study. Recent studies indicate that bacteria, including certain harmful strains of *S. aureus* can cause serious nosocomial or hospital acquired infections, but will simply die in a few hours when placed on cooper alloy surfaces at room temperature (Lewis 2005; Ozen et al. 2014b and Colak et al. 2015).

## CONCLUSIONS

With the rapid increase of world population, natural lands have been converted to areas of residence and this leads to many problems in nature. Synthetically produced coloring agents have adverse impacts on human life and nature and pose a significant threat to human life.

Within the context of the current research, it was aimed to develop a natural wooden surface material as an alternative to synthetic coloring agents by obtaining a natural dye from pomegranate fruit and to determine its antifungal and antimicrobial activities by administering it to wooden materials.

Among the treatment samples on which pomegranate skin coloring agent was administered, the most effective results against white decay (*Trametes versicolor*) fungus were obtained for Scotch pine tree with aluminium sulphate administered through ultrasonic method (yellow pine tree, 5.81 %) and for beech tree, the most effective results were obtained from the control samples (beech tree, 17.28 %). The most effective results against brown decay (*Postia placenta*) fungus were obtained from the control samples of beech tree treated with ultrasonic method (15.44 %) and for yellow pine tree, the most effective results were obtained from vinegar mordant implemented with ultrasonic method (6.46 %). Against brown decay fungus, all the results obtained for all the treatment samples are better than those obtained through synthetic dye.

In general, pomegranate skin and mordant complexes have yielded effective outcomes against the test microorganisms. The synthetic dye was found to have no antimicrobial activity. When the results are considered in general, it is seen that this study can make contribution to the development of natural dyes as alternative to synthetic dyes. This research is believed to provide a direction for future research. In short, natural dyes can become popular again to color and protect wooden materials.

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