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Antimicrobial Properties of Silk Fabrics Dyed with Green Walnut Shell (*Juglans regia* L.)

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Abstract

Natural dyes are obtained from plants, insects, lichens, fungus and molluscs which have been used since ancient times. These dyes have antimicrobial, anticarcinogenic and antihelmintic properties. Walnut (*Juglans regia* L.), a species of Juglandaceae family, is grown in Turkey. Green walnut shell has also antimicrobial functions due to its juglone components known as one of the strongest antimicrobial chemical compound. In this study, alum-mordanted and unmordanted silk fabrics were dyed with green walnut shell. Concentration was varied at 50, 100, 150 and 200 % owf (weight of fabric), in order to assess antimicrobial properties of green walnut shell. Pathogenic strains of *Staphylococcus aureus* subsp. *aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) were used to test for fabrics' antimicrobial activities. Reductions of bacterial growth were determined using AATCC test methods. All the fabrics which were dyed with green walnut shell indicated antimicrobial effect on both bacteria species. Antimicrobial effect was increased from 50 to 200 % owf (weight of fabric) dye concentration. These results indicated that unmordanted silk fabrics dyed with green walnut shell had antimicrobial effect as high as alum-mordanted silk fabrics dyed with green walnut shell and *S.aureus* were more sensitive than *E.coli*.

1. Introduction

Formerly, wool and silk fibres were always dyed with natural dyes extracted from renewable sources such as plants, crops, animals and minerals in nature [1]. They were the primary colour source of textiles until mid-to late 19th century [2]. Nowadays natural dyes have attracted renewed attention because of their bio-degradability, sustainable production and uncommon, soothing shades [3]. Natural dyes are non-allergenic, non-toxic to the human body and eco-friendly in comparison with synthetic ones; hence the usage of natural dyes in textile dyeing is getting increased day by day [4].

Natural dyeing began in China and Central Asia, then it was technically developed by Egyptians [5]. In particular, natural dyes were improved in India. Mesoamerican civilizations such as Olmecs, Mayas, Aztecs and Teotihuacans used natural substances for the dyeing production. The Maya used natural dyes comprising both organism-derived (mainly from plants) and inorganic dyes [6]. In the Ottoman Empire, natural dyes and dyehouses were very important. Natural dyes especially, *Rubia tinctorum* L. (madder), *Crocus sativus* (crocus), *Reseda luteola* L. (weld) and *Rhamnus petiolaris* Boiss. (buckthorn) were used in natural dyeing. Madder and buckthorn were commercial dyes which were imported. *Reseda luteola* L. was used in the thirteenth-century Seljuk carpets and from the fifteenth to twentieth-century, it was used in Ottoman textiles for yellow and green colours. *Rubia tinctorum* L. was also used in the Ottoman dyehouses in the XVII. century [7,8].

The textile industry is one of the most polluted sectors in the world. It contributes a great deal to poor labour conditions, non-renewable energy and waste water, contamination and environmental impact [9]. Synthetic dyes are highly cytotoxic and carcinogenic to mammalian cells and act as a liver tumour promoter. They can also decrease the food intake capacity, growth and fertility

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rates, cause damage to liver, spleen, kidney and heart; inflict lesions on skin, eyes, lungs and bones [10]. Alternatively, dyes obtained from plants, insects/animals and minerals are proven to have bio-degradability, sustainability, eco-friendliness and generally higher compatibility with the environment [11].

People have used natural dyes since ancient times for dyeing carpets, rugs and clothings by using roots, stems, barks, leaves, berries and flowers of various dye plants [12]. These dyes are reported as potent antimicrobial agents owing to the presence of a large amount of compounds such as anthraquinones, flavonoids, tannins, naphthoquinones etc. which possess strong antimicrobial properties [13].

Textile materials are often prone to attacked by microorganisms, as these provide basic requirements such as moisture, oxygen, nutrients and temperature for microbial growth and multiplication [14]. Antimicrobial finishes prevent the growth of microorganisms on fabrics used in wide variety of apparel, home furnishing, commercial and industrial products. Fabrics will have a longer life when treated with some type of antimicrobial finishes that reduce or prevent damage from microorganisms [15].

In the previous dyeing studies, wool, cotton and polyamide fabrics [16], wool fabrics [17] and polyamide fabrics [18] were used. In the present study, we used silk fabrics in the dyeing process. Silk fabrics were dyed with green walnut (*Juglans regia* L.) shell (Fig. 1) presence and absence alum mordant. *Juglans regia* L., among the large numbers of plants grown over the world, which is cultivated throughout southern Europe, northern Africa, eastern Asia, USA and western South America [16]. The parts of a walnut tree such as leaves, husk and shell are tested as potential dyeing materials for different textile substrates. Textile materials dyed with aqueous green walnut shell extracts yield brown colour shades.

The aim of this study is to assess antimicrobial activity of silk fabrics dyed with green walnut shell against pathogenic strains of *Staphylococcus aureus* subsp. *aureus* and *Escherichia coli*.

Figure 1. Green walnut shell is used as a natural dyestuff

2. Materials and Methods

2.1. Materials

Commercially silk fabrics were provided by Armaggan Company. Green walnut shell was collected from Dardanos, Çanakkale in Turkey, by TCF-Armaggan, Cultural Heritage Preservation and Natural Dyes Laboratory. Alum mordant [$KAl(SO_4)_2$.12H2O], Nutrient Agar and Nutrient Broth were purchased from Merck (Germany).

To investigate antimicrobial activity of green walnut shell on alum mordanted and unmordanted silk fabrics, Gram-positive bacteria *Staphylococcus aureus* subsp. *aureus* (ATCC 29213), which is a clinical isolate that is obtained from wounds and Gram-negative bacteria *Escherichia coli* (ATCC 25922), which is a member of gut microbiota that cause urinary tract infections, were used for evaluation. Antimicrobial activities were tested according to AATCC (American Association of Textile Chemists and Colorists) Standards.

2.2. Mordanting and Dyeing Process

The silk fabrics were cut in 30 x 30 cm^2 size for five alum-mordanted fabrics and five unmordanted fabrics. Potassium aluminium sulphate (alum) [KAl(SO4)2.12H2O] was used as a mordant. Five of silk fabrics were treated with 10 % alum, at 65 °C at 4.5 pH for 1 hour in a water bath. Then fabrics left in mordant solutions for overnight. After this stage, fabrics were taken into dyeing bath.

Dried green walnut shell rinds were used at 50, 100, 150 and 200 % owf (weight of fabric) for dyeing. Green walnut shell aqueous extracts were obtained at 80 °C. Mordanted and unmordanted silk fabrics were dyed at 65 °C at 1:100 (material liquor ratio) at 6.0 pH and were stirred well for 60 min. After dyeing method, silk fabrics were washed with cold water and dried.

Several test methods have been developed to evaluate the antimicrobial activities of textiles. These methods fall into two categories: qualitative and quantitative test methods [19].

In Parallel Streak Method (AATCC Test Method 147-2004), broth cultures of *S.aureus* and *E.coli* were developed at 37 °C for 24 hours. 1 loop of culture was inoculated sterilized agar plates by making 7.5 cm five parallel streak and then sterilized fabrics which were cut in 25 x 50 mm, were pressed onto the agar plates. After incubation at 37 ± 2°C for 18-24 hours, a clear zone of interrupted growth around and underneath of the testfabrics were indicated antibacterial activity of green walnut shell.

In Antimicrobial Finishes on Textile Material (AATCC Test Method 100-2012) Method, *S.aureus* and *E.coli* test microorganisms were incubated on the Nutrient Agar Medium at 37 °C for 24 hours and then bacterial suspensions which containing 106 CFU/ml, were prepared in saline buffer. The circular fabric samples were cut 480 mm in diameter, placed in containers and sterilized at 121°C for 20 min. After the sterilization, 1 ml bacterial suspension were dropped centre of the sterilized fabrics. Then neutralization broth were added onto the fabrics and containers were shaken well with vortex for 1 min. Serial dilutions were made. 100 µL were taken from the solution and inoculated onto the Nutrient Agar Medium plates. The Petri plates were incubated at 37 ± 2°C for 18-24 hours. After the incubation viable colonies were counted (according to 104 dilutions) and expressed as a percentage reduction according to the Eq. (1) below:

$$\% \text{ Reduction} = [(B-A)/ B] \times 100 \quad (1)$$

Where, A is the number of bacteria colonies of treated fabrics, and B is the number of bacteria colonies of untreated fabrics.

3. Results and Discussion

In the present study, according to Parallel Streak Test Method, clear zones of interrupted growth underneath of the test fabrics had been obtained. All the fabrics (mordanted and unmordanted) which were dyed with green walnut shell concentration was varied at 50, 100, 150 and 200 % owf (weight of fabric), indicated antimicrobial effect to both of bacteria. Antimicrobial effect were increased from 50 to 200 % owf dye concentration. Unmordanted silk fabrics had between 89.68 and 99.81 % antimicrobial activities against *S.aureus* (Fig. 2) and between 70.37 and 93.85 % antimicrobial activities against *E.coli* (Fig. 3).

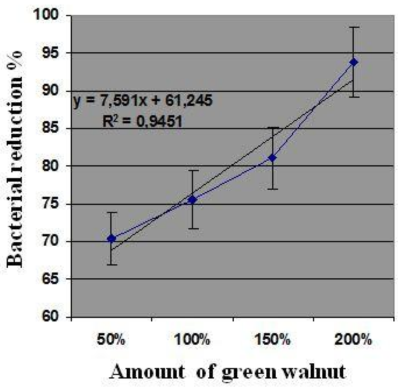

Figure 2. Amount of green walnut shell and *S.aureus* reduction (%) on mordanted silk fabric

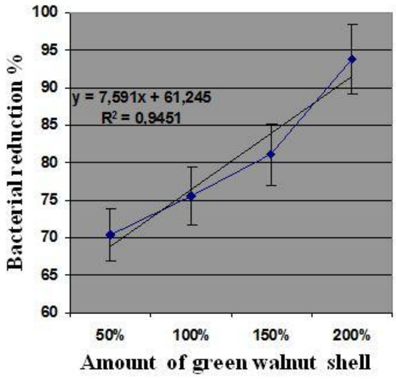

Figure 3. Amount of green walnut shell and *E.coli* reduction (%) on unmordanted silk fabric

Antimicrobial activities against *S.aureus* alum-mordanted silk fabrics range from 90.87 to 99.43 % (Fig. 4) and antimicrobial activities against *E.coli* of alum-mordanted silk fabrics range from 69.30 to 82.56 % (Fig. 5).

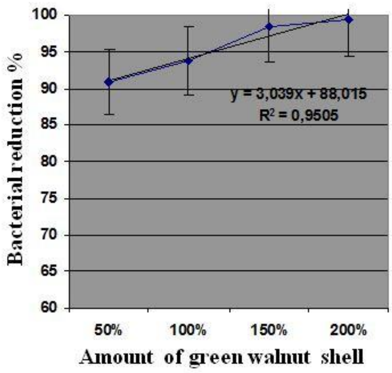

Figure 4. Amount of green walnut shell and *S.aureus* reduction (%) on mordanted silk fabric

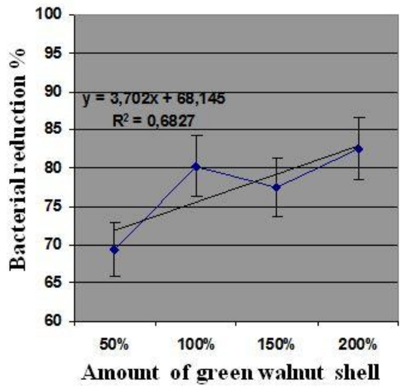

Figure 5. Amount of green walnut shell and *E.coli* reduction (%) on mordanted silk fabric

These results indicated that unmordanted silk fabrics had antimicrobial effect as high as alum-mordanted silk fabrics and *S.aureus* were more sensitive than *E.coli* against green walnut shell extract.

Ghaheh et al. reported similar results using walnut shell extract and pomegranate. Unmordanted and mordanted with chrome, copper (II) sulphate, iron (II) sulphate, iron (III) sulphate, aluminium sulphate, tin (II) chloride, potassium dichromate and tannic acid wool fabrics dyed with walnut shell extract and pomegranate showed antibacterial properties against *S.aureus*, *E.coli* and *P.aeruginosa* [17]. In the present study, alum-mordanted and unmordanted silk fabrics dyed with green walnut shell extract showed antibacterial activities against *S.aureus* and *E.coli*. Mirjalili et al. screened that polyamide fabrics with 1, 3, 5 and 10 % owf were dyed with green walnut shell showed better efficiency against *E.coli* in comparison with *S.aureus. S.aureus* had better antibacterial activity with using mordant. The present study indicated that unmordanted silk fabrics dyed with green walnut shell had antimicrobial effect as high as alum-mordanted silk fabrics dyed with green walnut shell against *S.aureus*. The antibacterial activity of dyed fabrics was ranked as ferric sulphate > cupric sulphate > potassium aluminium sulphate > without mordant against *S.aureus* and cupric sulphate > ferric sulphate > potassium aluminium sulphate > without mordant against *E.coli* [18]. Jabli et al. examined antimicrobial potential of stem and leaves methanolic extracts of Tunisian *Juglans regia* L. (walnut). Wool, cotton and polyamide fabrics dyed with stem, leaves and their mixture were performed against Gram (+) bacteria, Gram (-) bacteria and mold using disc diffusion method. Results indicated that *Aspergillus niger* and *Salmonella arizonae* 1 were the most sensitive microorganisms showing the largest inhibition zones (18 < d <20 mm) [16]. Jabli et al. performed the walnut stem and leaves methanolic extracts, in the present study, green walnut shell aqueous extracts were examined against Gram (+) bacteria *S.aureus* and Gram (-) bacteria *E coli*.

4. Conclusion

Present results indicate that green walnut shell extract can be used for silk fabric dyeing with or without mordant. Also, it has an antimicrobial effect against *S.aureus* and *E.coli*. Further research is needed on antimicrobial textile products such as baby clothes, socks, underwears, bedclothes, bathrobes, towels, hospital materials (aprons, bonnets and surgical garments) etc. Moreover, patients have some diseases such as skin allergy, eczema, psoriasis, fungal infectious disease, heat rash and acne. Therefore they can use textiles dyed with green walnut shell extract rather than synthetic dyed textiles. We can suggest green walnut shell extract as a dyeing material. It can be an alternative natural source for health and the environment.

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