Antimicrobial Activities of Some Natural Dyes and Dyed Wool Yarn

Comlekcioglu, Nazan*+; Aygan, Ashabil; Kutlu, Mehtap
Kahramanmaras Sutcu Imam University, Science and Letters Faculty, Biology Department, Kahramanmaras, TURKEY

Kocabas, Yusuf Z.
Kahramanmaras Sutcu Imam University, Turkoglu Vocational High School, Medicinal Plants Department, Kahramanmaras, TURKEY

ABSTRACT: *Natural dyes gained increasing attention due to environmental considerations. Many of the dye plants have also medicinal values. This study aimed to evaluate the effect of 25 plants as dye and their dyed wool yarns against microorganisms. Prokaryotes were found to be more sensitive to dye extracts than eukaryotic microorganisms and dyestuff from Punica granatum, Berberis vulgaris, Agrimonia eupatoria, Rhus coriaria were effective against all bacteria. Sarcina lutea, Bacillus subtilis, MRSA and Enterococcus faecalis were sensitive to almost all dye extracts even at low concentrations. The dyed wool material tested with microorganisms, and maximum inhibition rates were obtained against S. lutea and MRSA of wool samples dyed with P. granatum and R. coriaria, respectively, while there was a drastic decrease in E. faecalis growth with the A. cepa and R. petiolaris.*

KEYWORDS: *Anatolia; Antimicrobial; Dyeing; Plants; Natural dyes; Wool yarn.*

INTRODUCTION

Interest in natural dyes among the people dates back to ancient times and natural dyes were the main colorants available for textile dyeing procedures until the end of nineteenth century [1] (*Bechtold et al.* 2003). After the development of synthetic dyes, the use of natural dyes for textile dyeing almost disappeared [2] (*Bechtold et al.* 2007). Recently, there has been increasing interest on the application of natural dyes in textile dyeing as a result of their lower toxicity and allergic reactions [3] (*Samantha & Agarwal* 2009). Natural dyes have also become more acceptable to environmentally conscious people on the account of their high compatibility with the environment [4] (*Deo & Desai* 1999).

Textiles are getting important in human life by being a mediator agent for microorganisms and this case imposes a number of undesirable effects. Pathogenic or odour-generating bacteria and moulds could find themself in a perfect condition for their growth especially in skin contact [5] (*Shahid et al.* 2013. Today's customers prefer more comfortable, hygienic and odourless product in use. Therefore textile materials with antimicrobial properties gained an increasing attention [6] (*Khan et al.* 2011).

* *To whom correspondence should be addressed.*
\+ *E-mail: noktem@ksu.edu.tr*
1021-9986/2017/2/137-144 6/$/5.60

The fabrics bearing antimicrobial property is also preferred in sportswear and hotels as well as in medical area such as masks, hospital covers, surgical gowns [7] (*Dev et al.,* 2009). In hospital, the textile materials used can cause serious infection by transmitting the hospital microorganisms. Plants bearing antimicrobial properties are getting focus of interest due to problems emerged from the misused antibiotics. Plants synthesize many secondary metabolites having antimicrobial activity as a consequence of their development and growth. The people world wide used many traditional medicinal plants for ages. Compounds able to limit or destroy the microbial agents without toxicity to host organism are the most prominent substances of the battle for better life.

Pigments are already used in various field such as medicine, food, paper, textiles, animal feed etc. If these dye pigment have an antimicrobial effect, it would be a desired feature for quality of life. The plants are still remains as an important source of natural dyes although superior position of the synthetic dyes. Natural dyes can also inhibit the microorganisms [8] (*Han & Yang* 2005), and many of the plants used in dyeing also known with their medicinal properties. Some of these dye-yielding plants have been reported for their significant antimicrobial effects. Dyes from *Mallotus philippinensis* [9] (*Gupta et al.* 2004), *Punica granatum* [9,10] (*Gupta et al.*, 2004, *Calis et al.* 2009), *Quercus infectoria* [9,11,12] (*Gupta et al.* 2004, Singh et al. 2005, *Gupta and Laha* 2007), *Acacia catechu* [11] (Singh et al. 2005), *Rheum emodi* L. [13] (*Khan et al.* 2012) displayed activity against some gram positive and gram negative bacteria.

Natural dyes obtained from plants were also widely used in Anatolia throughout history [10] (*Calis et al.* 2009). In the present study, antimicrobial effects of 25 plants, which are known as traditional dye sources in Turkey, have been examined against nine microorganisms. Water was used for the extraction of natural dye from the plants. Antimicrobial activity and Minimum Inhibition Concentration (MIC) of dyestuff was investigated and antimicrobial effect of wool yarn treated with selected dyes were also determined against test microorganisms.

EXPERIMENTAL SECTION

Plant samples

The names, parts and the expected colors of the plants used in this study were given in Table 1. Some of the plant materials were obtained from the native herb markets and the others collected from their natural habitats in April 2014. The plants were immediately air-dried and the experiments were carried out in 2014. Plant samples were dried in an incubator at 40 ºC for 12 h and stored at room temperature in sealed bags until use.

Microorganisms and Culture Conditions

The microorganisms were obtained from the culture collection of Biotechnology Laboratory, Kahramanmaras Sutcu Imam University. *Escherichia coli*, *Sarcina lutea*, *Proteus vulgaris*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Saccharomyces cerevisiae* and *Candida albicans* were used as test microorganisms and maintained on nutrient agar (Composition: beef extract 1.5 g; yeast extract 1.5 g; peptone 5.0 g; NaCl 5.0 g; agar-agar 15 g; pH 7.5) and Sabouraud dextrose agar (Composition: peptone 10 g; dextrose 20 g; agar-agar 15 g; pH 5.6) at 4 ºC. For activation of microbial growth, 5 mL of nutrient broth and Sabouraud dextrose broth were inoculated with stock culture samples and incubated at 30-37 ºC, 150 rpm for 12 h.

Extraction of dyestuff and dyeing

Dried plant samples were ground into fine particles by using laboratory blender (Waring). For the dye extraction, 10 g of each ground material were added to bottles containing 100 mL of distilled water. Then the tubes were immersed in a boiling water bath for 60 min. After removing the plant materials by filtration, the extract was clarified by centrifugation at 2000 g for 10 min.

The raw wool yarn (unbleached) was used for the dyeing experiments. They were soaked in 250 mL tap water for 30 min before the dyeing processes. Prior to dyeing, the wool yarn was pretreated with $KAl(SO_4)_2 \cdot 12H_2O$ (1%, w/v) as mordant at a temperature of 90 ºC for 60 min. Then the dyeing was carried out by soaking the wool yarn in dye extracts at 90 ºC for 60 min. Finally, the yarns were rinsed with cold water to remove the unbound dyestuff and dried at 30 ºC in shadow.

Determination of antimicrobial activity and MICs of dyestuff

The antimicrobial activities of extracted dyestuff were determined by the well-diffusion method. Mueller Hinton agar plates were cultured with a standardized inoculums

Table 1: Plants used for dye extraction in this study.

Botanical Name	Local Name	Used Parts	Expected Colors
Rhamnus petiolaris	Cehri	Fruit	Yellow-orange
Juglans regia	Ceviz	Green fruit peel	Brown
Laurus nobilis	Defne	Leaf	Light yellow
Erica manipuliflora	Funda	Above ground	Brown, yellow
Vitex agnus-castus	Hayıt	Leaf	Light brown, greenish
Juniperus foetidissima	Kokulu ardıç	Leaf	Light yellow
Juniperus excelsa	Boylu ardıç	Leaf	Light yellow
Berberis vulgaris	Kadın tuzluğu	Fruit	Yellow-Orange
Lawsonia inermis	Kına	Leaf	Red, Brown
Agrimonia eupatoria	Koyunotu	Leaf	Yellow
Cistus creticus	Laden	Leaf	Brown,Yellow
Reseda lutea	Muhabbet çiçeği	Flower	Yellow
Sambucus nigra	Mürver	Leaf	Yellow
Punica granatum	Nar	Fruit peel	Yellow
Eucalyptus globulus	Okaliptus	Leaf	Hardal sarısı
Matricaria chamomilla L	Papatya	Flower	Yellow
Pinus brutia	Kızıl çam	Bark	Brown
Platanus orientalis	Doğu çınarı	Bark	Red
Cartamus tinctorius L.	Aspir	Flower	Yellow
Salvia officinalis	Adaçayı	Leaf	Yellow-orange, green
Verbascum orientale All.	Sığırkuyruğu	Leaf	Yellow
Allium cepa	Soğan	Dry outer leaf	Yellow-orange
Rhus coriaria	Sumak	Flower	Yellow, brown
Curcuma longa	Zerdeçal	Flower	Yellow
Olea europaea	Zeytin	Leaf	Yellow-green

(10^8 cfu/mL) of each bacterial strain and also Sabouraud dextrose agar were cultured with each of yeast strains (2.1 x 10^3 cfu/mL) [14] (*Collins et al.* 1989). On the inoculated agar plates, the wells were prepared with a cork borer 6 mm in diameter and 100 µL of dye extract, which is used for dying process; were loaded. The plates were incubated at 30 ºC and 37 ºC for 24 h for fungal strain and bacterial strain, respectively. After incubation, the diameter of inhibition zones was measured by a compass. Distilled water (dH_2O) was used as solvent control since it was used as a solvent for extraction. The dye extracts showing antimicrobial activity were then tested to determine the MIC values.

Dye extracts were evaporated at 50 ºC under vacuum and dissolved in Mueller Hinton broth to obtain the final concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v). After sterilization of the medium, test microorganisms were inoculated and allowed to incubation overnight. MICs were determined as the lowest concentration of dyestuff inhibiting the visible growth of each microorganism in tube.

Determination of antimicrobial activity of dyed wool yarn

The 1 g of dyed wool yarn was introduced into the 20 mL of nutrient broth and Sabouraud dextrose broth. Media were inoculated with the respective microbial

Table 2: The antimicrobial activity of natural dyestuff against test microorganisms*.

Dye source	*E. coli*	*S. lutea*	*K. pneumoniae*	*B. subtilis*	*MRSA*	*E. faecalis*	*P. vulgaris*	*S. cerevisia*	*C. albicans*
Rhamnus petiolaris	-	+	-	+	+	+	-	-	-
Juglans regia	-	+	-	+	-	+	-	-	-
Laurus nobilis	-	-	+	+	+	-	-	-	-
Erica manipuliflora	-	+	-	+	+	+	-	-	-
Vitex agnus-castus	-	-	-	-	-	+	-	-	-
Juniperus foetidissima	-	+	-	+	+	+	+	-	-
Juniperus excelsa	-	+	-	+	+	+	+	-	-
Berberis vulgaris	+	+	+	+	+	+	+	-	-
Lawsonia inermis	+	+	-	+	+	+	+	-	-
Agrimonia eupatoria	+	+	+	+	+	+	+	-	-
Cistus creticus	+	+	-	+	+	+	+	-	-
Reseda lutea	-	-	-	-	-	-	-	-	-
Sambucus nigra	-	-	-	-	-	-	-	-	-
Punica granatum	+	+	+	+	+	+	+	+	-
Eucalyptus globulus	+	+	-	+	+	+	+	+	-
Pinus brutia	-	+	-	+	+	+	+	-	-
Platanus orientalis	-	-	-	-	-	-	-	-	-
Salvia officinalis	-	+	-	+	+	-	-	-	-
Allium cepa	-	+	+	+	+	+	+	+	-
Ruhus coriaria	+	+	+	+	+	+	+	-	-
Olea europaea	-	-	-	+	+	+	-	-	

** (+) Inhibition, (-) No-inhibition*

strains followed by overnight incubation at 37 ºC and 30 ºC. Control groups were also conducted with un-dyed yarn and yarn with mordant. The cultures were thoroughly shaken in order to bring microorganisms into suspension and growth of microbial strains in all tubes were determined spectrophotometrically (OD_{660}). Uninoculated sterile medium was used as blank [15] (*Prusty et al.* 2010). All experiments and controls were conducted in triplicates and the mean values were used.

RESULTS AND DISCUSSION

Knowledge about natural dyes is mainly come from the literature of traditional dyeing prior to the use of synthetic dye [16] (*Hill* 1997). Fifty taxa of dye plants were reported for East Anatolia [17] (*Ozgokce & Yilmaz* 2003), while *Doğan et al.* [18] (2004) listed 123 taxa for Turkey, and several of them are endemic species. The studied plants were used for dyeing traditionally in Anatolia, and some of these plants were also known for their medicinal properties. In this study, antimicrobial activities of several dye plants, which are known in Anatolia, were studied and many of them showed antimicrobial effects against tested microorganisms. The experiments were carried out for dye extraction from 21 different plant species using water as solvent. Extracted natural dyes were screened for their antimicrobial activity against selected microorganisms and results are given in Table 2. Screening studies showed that dyestuff from *P. granatum, B. vulgaris*, *A. eupatoria*, *R. coriaria* were effective against all bacteria and none of the dyes had antimicrobial activity on *C. albicans.* However *S. cerevisia* was sensitive to *P. granatum, E. globules* and

Table 3: MIC values of natural dyes extracted from plantal samples.

Dye source	*E. coli*	*S. lutea*	*K.pneumonia*	*B.subtilis*	*MRSA*	*E. faecalis*	*Proteus sp.*	*S. cerevisia*
Rhamnus petiolaris	-	2	-	4	4	8	-	-
Juglans regia	-	0.5	-	1	-	4	-	-
Laurus nobilis	-	-	0.25	8	16	-	-	-
Erica manipuliflora	-	0.156	-	5	0.625	1.25	-	-
Vitex agnus-castus	-	-	-	-	-	1	-	-
Juniperus foetidissima	-	16	-	4	0.25	1	8	-
Juniperus excelsa	-	*	-	4	1	1	16	-
Berberis vulgaris	13	3.25	*	3.25	0.101	0.325	6.5	-
Lawsonia inermis	16	4	-	4	2	*	16	-
Agrimonia eupatoria	*	0.125	16	1	1	8	*	-
Cistus creticus	*	0.406	-	1.625	0.812	0.406	*	-
Punica granatum	16	1	*	1	0,5	0.125	16	*
Eucalyptus globulus	*	2	-	2	1	1	16	*
Pinus brutia	-	0.0675	-	0.5	0.5	4	4	-
Salvia officinalis	-	0.5	-	0.125	0.25	-	-	-
Allium cepa	-	0.5	16	4	1	4	4	*
Rhus coriaria	*	1	8	2	2	16	4	-
Olea europaea	-	-	-	2	0.125	16	-	-

** MIC values more than 16 mg/mL*

A. cepa. The dye extracts of *V. agnus-castus* had no activity against all test microorganisms except *E. faecalis*. According to the results, prokaryotes were found to be more sensitive to dye extracts than eukaryotic microorganisms. No activity was observed with *R. lutea*, *S. nigra* and *P.orientalis*, but some antimicrobial properties were reported for these plants [19,20,21] (*Kumarasamy et al*. 2002, *Hearts et al.* 2010, *Jing* 2011). This could be because of the differences in used parts, the extraction methods, and tested microorganisms between laboratories.

The MIC values of the dye extracts showing antimicrobial activity were tested at five different concentrations. The highest antimicrobial activity was found with *P. brutia* on *S.lutea*. Most of the dye extracts were not effective on clinic isolate *E. coli* and which was confirmed by MIC values. So it could be concluded that *E. coli* was the most resistant bacteria for these extracts. *S. lutea*, *B. subtilis*, MRSA and *E. faecalis* were sensitive to nearly all dye extracts even at low concentrations. *C.albicans* was not inhibited with all tested concentrations of extracts. The minimum inhibition concentration (mg/mL) of active samples was studied further and results are given in Table 3.

Antimicrobial activity of a dye will vary while it is in solution or it is held by wool yarn [11] (*Singh et al.*, 2009). Results given above showed antimicrobial effect of dyes in a growth media against selected microorganisms and for the evaluation of their effectiveness, it is necessary to study the antimicrobial activity of dyed yarn (wool). Therefore, the antimicrobial activity of dyed wool yarns was tested in the next step of experiments. Bacteria showed good growth with untreated (no dye and no mordant) wool, indicating that the wool does not inhibit bacteria by itself. To eliminate the effect of mordant, previously the wool was treated with mordant and bacterial growth in the presence of mordant treated yarn was taken 100% as control group.

Table 4: Effects of dyed wool yarn on bacterial growth (%).

Dye source	*E. coli*	*S. lutea*	*K.pneumonia*	*B.subtilis*	*MRSA*	*E. faecalis*	*Proteus sp.*
Rhamnus petiolaris	*	42.37 ± 6.25	*	70.81 ± 3.34	56.84 ± 3.93	23.58 ± 1.31	*
Juglans regia	*	-	*	-	*	30.05 ± 2.62	*
Laurus nobilis	*	*	98.88 ± 7.19	-	78.65 ± 5.310	*	*
Erica manipuliflora	*	-	*	-	98.35 ± 10.78	-	*
Juniperus foetidissima	*	-	*	-	52.58 ± 7.600	-	81.01 ± 1.48
Juniperus excelsa	85.14 ± 4.41	-	*	-	85.19 ± 10.20	-	85.19 ± 7.21
Berberis vulgaris	93.99 ± 3.25	39.74 ± 8.32	88.62 ± 5.28	-	87.93 ± 7.960	-	79.34 ± 2.01
Lawsonia inermis	86.04 ± 1.20	38.60 ± 17.75	*	76.91 ± 10.39	51.71 ± 11.67	39.76 ± 1.740	69.75 ± 2.80
Agrimonia eupatoria	90.14 ± 1.86	-	93.69 ± 0.81	-	60.59 ± 13.74	-	76.29 ± 4.07
Cistus creticus	88.03 ± 2.41	-	*	-	-	-	75.24 ± 3.55
Punica granatum	-	22.54 ± 2.52	89.87 ± 8.06	-	63.51 ± 15.07	30.59 ± 0.740	66.92 ± 1.45
Eucalyptus globulus	*	-	*	-	51.49 ± 11.98	-	72.22 ± 2.87
Pinus brutia	*	91.32 ± 6.08	*	-	70.46 ± 9.690	74.47 ± 7.790	86.75 ± 5.15
Salvia officinalis	*	-	*	35.62 ± 1.32	77.41 ± 11.94	*	*
Allium cepa	*	83.68 ± 7.71	65.90 ± 4.56	-	78.60 ± 6.800	26.20 ± 3.250	-
Rhus coriaria	93.26 ± 5.57	41.58 ± 6.32	65.35 ± 2.81	-	27.25 ± 8.830	38.87 ± 2.180	64.12 ± 1.62
Olea europaea	*	*	*	-	-	-	*

** Not tested, - tested and no inhibition observed*

Dye extracts those are not showing antimicrobial effects were not tested. The results of antimicrobial effects of dyed yarn were given in Table 4. *J. regia* showed antimicrobial effect only on *E. faecalis*, and the bacterial growth was determined as 30%. Maximum inhibition rates were obtained against *S. lutea* and MRSA of wool samples dyed with *P. granatum* and *R. coriaria*, respectively, while there was a drastic decrease in *E. faecalis* growth with the *A. cepa* and *R. petiolaris*. *P. granatum*, *R. petiolaris*, *A. cepa* and *R. coriaria* were found to be the most efficient antimicrobial agents on wool yarn against certain microorganisms. Bacterial growth rates of 22.54-89.87%, 23.58-70.81%, 26.20-83.68% and 27.25-93.26% were observed on wool dyed with *P. granatum*, *R. petiolaris*, *A. cepa* and *R. coriaria*, respectively.

Water extract of *P. granatum* rind dyed wool yarn into yellow color and its dyestuff was found to be the most effective antimicrobial against test microorganisms. *P. granatum* has been used to dye wool yarn in yellow, orange, dark brown and black colors in Anatolia [22] (*Kurt & Sahin* 2013). *P. granatum* contains a large amount of tannin and is reported as a potent antimicrobial agent [11] (*Singh et al.* 2005). *Gupta et al.* [9] (2004) found that the dye solution of *P. granatum* displayed activity against *E. coli*, *K. pneumoniae* and *P. vulgaris*. [10] (*Calıs et al.* 2009) reported an inhibition of *B. subtilis* growth on wool samples dyed with *P. granatum*, however no antimicrobial effect of wool yarn dyed with *P. granatum* was found against *B. subtilis*, in this study. Wool yarn was dyed with *R. petiolaris* into yellow color and *E. faecalis* growth was highly inhibited by this dyed wool yarn. Endemic plant *R. petiolaris* [23] (*Iskender et al.* 2006) has rich flavonoid content and important for natural dyeing due to their yellow dyestuff [24] (*Deveoglu et al.* 2009). To the best of our knowledge, antimicrobial properties of wool yarn dyed with *R. petiolaris* were studied firstly in this study. The color obtained from *A. cepa* was brown and the dyed wool yarn was found to be effective on *E. faecalis*. The dry skin of onion produces natural dye which has been used for dyeing textiles [25] (*Vankar et al.* 2009). *Calis et al.* [10] (2009) reported antimicrobial activity for a wool sample dyed with *A. cepa* against *B. cereus*, *B. subtilis*, *P. aeruginosa* and *S. epidermidis*. *Datta et al.* [26] also stated that cotton fabrics dyed with onion skin had similar antimicrobial activity against both Gram positive and negative bacteria. Although *R. coriaria* is used to dye traditional rugs and carpets into yellow, green, brown and grey [18, 27] (*Dogan et al.* 2004, *Sanlı* 2011), in this study, beige color was obtained from *R. coriaria* fruits. Maximum antimicrobal activity of dyed wool samples was observed on MRSA and *E. faecalis*.

CONCLUSIONS

Ecological issues are becoming serious subjects in the industrial production and application processes. Synthetic dyes are known to be an important part of the environmental pollution. This is because the chemicals used in dyeing have several carcinogenic features and can cause allergy in humans. Therefore, there has been an increase in the research of natural dyes. Researchers focused on the cases such as coloring material, extraction and dyeing properties, fastness characteristics and antimicrobial efficiency. Usually in textile industry, *E.coli* and *S.aureus* are the common microorganisms for determination of antimicrobial property of fabrics. Although majority of the yarn dyed with plant extract did not produced an antibacterial effect against *E.coli*, nearly all yarns dyed were effective on *S.aureus* (Table 4). *Staphylococci* species are the most common member of skin habitat. Normally these microorganisms are occasionally pathogenic, but MRSA (*Methicillin resistant S.aureus*) is one of the most important nosocomial infection causing microorganisms. Therefore these dye extracts could be very promising for textile industry. However, additional studies are needed to state the usefulness of natural dyes.

Received : Feb. 11, 2016 ; Accepted : Dec. 26, 2016

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