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Short Communication

Antimicrobial effect of natural dyes on some pathogenic bacteria

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In this study we have detected the antimicrobial activity of four natural dyes (obtained from *Rubia tinctorum, Allium cepa, Punica granatum* L and *Mentha* sp.) on *Staphylococcus aureus* ATCC 25923, *Shigella sonnei* RSKK 877, *Escherichia coli* ATCC 35218, *Bacillus megaterium* RSKK 5117, *Bacillus subtilis* RSKK 244, *Bacillus cereus* RSKK 863, *Pseudomonas aeruginosa* ATCC 29212, *Streptococcus epidermidis*, *Salmonella* 21.3 and *P. aeruginosa* ATCC 27853. *P. granatum* dye was most effective against the test bacteria except *E. coli* and *S. epidermidis*. The textile material impregnated with four natural dyes and maximum inhibition rates (respectivelly, 80, 86, 52%) were obtained against *B. subtilis* of wool samples dyed with *P. granatum*, *A. cepa* and *R. tinctorum* while maximum inhibition rates (91%) was found against *P. aeruginosa* of wool sample dyed with *R. tinctorum.*

Key words: Antimicrobial activity, natural dyes, textile.

INTRODUCTION

In recent decades, there has been an increasing tendency towards the prevention of microbial attack on textiles (Han and Yang, 2005). Natural fibres have keratin and cellulose, etc., that provide important requirements such as oxygen, moisture, nutrients and temperature for the bacterial growth (Singh et al., 2005). A variety of antimicrobial textile agents are reported such as organo-metalics, phenols, quaternary ammonium salts and organo-silicones (Yang et al., 2000). Synthetic compounds are more complex and it will take a long time for them to complete their natural cycles and return to nature; thus causing a lot of environmental pollution. Due to the fact that natural dyes can often inhibit the growth of microorganisms traditionally, different plants have been used as natural dyes in textile and carpet industries and it is believed that these dyes are less allergic and more stable than the chemical ones (Mehrabian et al., 2000).

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 centre Asia and Anatolia throught history. *Rubia tinctorium* L. is known for dyestufs which orginate from root material of field grown madder including anthraquinone derivates, the most important one is alizarin (Merck, 1996). It is known that anthraquinone derivates have been used as antiinflammatory, antimicrobic, antibacterial and anti-diuretic drugs (Swain, 1996). *Punica granatum* dye and many other common natural dyes are reported as potent antimicrobial agents owing to the presence of a large amount of tannins (Machado et al., 2002). *Mentha piperita* L. contains α-terpinene, isomenthone, menthol, trans-carveol,pipertitinone oxide, β-caryophyllene, carvone and limonene (Yadegarinia et al 2006). Flavanoids are a second class of health enhancing compound produced by *Allium cepa* L., with quercetin as an example. Flavanoids are active against microorganisms (Ekwenye et al., 2005)

At present, many of the plants used for dye extraction are classified as medicinal, and some of these have been shown to posses significantly antimicrobial effect. The antimicrobial activity of some of these dyes are reported as potent owing to the existence of phenol, tannin and quinone in their extracts. The antimicrobial effects of some plants used in dye industries contribute to the longer life of the products they are used in (Hussein et al., 1997, Mehrabian et al., 2000).

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The purpose of this research is to study the antimicrobial effect of four commercially available dye powders, obtained from *R. tinctorum* L., *A. cepa* L., *P. granatum* L., *M. piperita* L. against some common microbes. We also focused on the antimicrobial activity of wool fabric treated with these naturel dyes.

EXPERIMENTAL

Naturel dyes

The optimized commercial naturel dye powders of *R. tinctorum* L., *A. cepa* L., *P. granatum* L. and *M. piperita* L. were obtained from Mugla and Nigde, Turkey.

Naturel dyes application

To prepare aqueous dye solutions of these plants, 10 g of each of the powders were added to 300 ml of distilled water and boiled for 60 min 100^{o}C. The hot solution was filtered and any plant material left in the filter paper were discarded.

Wool was dyed by the standard method prescribed for natural dyes. The dyeing was carried out at 1:30 MLR (material to liquor ratio), for 30 min at 80^{o}C at neutral pH. Dyed samples were further treated with the aluminum potassium sulfate ($KA_l(SO4)_2 \cdot 12\ H_2O$) as mordant (0.5 gl^{-1}) at 60^{o}C for 20 min, and rinsed in hot and then cold water.

Test bacteria

Cultures of following microorganisms were used in the study: *Bacillus megaterium* RSKK 5117, *B. megaterium* RSKK 5117, *B. subtilis* RSKK 244, *P. aeruginosa* ATCC 29212, *S. epidermidis, B. cereus* RSKK 863, *P. aeruginosa* ATCC 27853, *S. sonnei* RSKK 877, *Salmonella* sp. 21.3, *S. aureus* ATCC 25923, *E. coli* ATCC 35218.

Antimicrobial screening test

Susceptibility of the bacterial strains to the natural dyes was investigated using the disc diffusion method (NCCLS, 1997). The culture suspensions were prepared and adjusted against 1 McFarland turbidity standard tubes. Muller Hinton Agar medium (15 mL) was poured into each sterile petri dish. Then the surface of the agar medium dish was inoculated with 100 µl cultures. Dyes were sterilized by filtration through 0.45 µm membrane filters. Empty sterilized discs of 6 mm were each impregnated with 100 µl natural dyes. Discs were placed on agar plates, and the plates were incubated at 37^{o}C for 24 h. The inhibition zones formed on the medium were evaluated in mm. All experiments were performed in duplicate.

In the next set of experiments the antimicrobial activity of dyed wool specimens was tested. The 1 g fabric (dyed and undyed) was introduced into the 100 ml nutrient broth inoculated with the desired microbe and incubated at 37^{o}C overnight for 16 h. The reduction of bacterial growth by dye was expressed as follows:

$$R = 100(A-B)/A$$

Where R = % reduction in bacterial population; A = absorbance (660 nm) of the media inoculated with bacteria and undyed fabric; B = absorbance (660 nm) of the media inoculated with bacteria and dyed fabric (Singh et al., 2005).

RESULTS AND DISCUSSION

Antimicrobial activity of natural dyes in solution

Four natural dyes were screened for their antimicrobial activity against ten test bacteria and the first screening showed that *P. granatum* was effective against most of the test bacteria except *E. coli* and *S. epidermidis*. *Mentha* sp. was effective against six test bacteria (*B. megaterium*, *B. subtilis*, *P. aeruginosa*, *S. epidermidis*, *B. cereus*). *R. tinctorum* has shown inhibition effect against *B. megaterium*, *S. aureus*, and *B. subtilis*. *A. cepa* exhibited activity against only two *Bacillus* strain (Table 1).

Singh et al. (2005) investigated antimicrobial activity of four natural dyes against some microorganisms and they found that *Quercus infectoria* was effective against all the test microorganisms (Singh et al., 2005). Other researches studied antimicrobial properties of eleven natural dyes against three types of gram-negative bacteria and they reported that seven of the dyes showed activity against one or more of the bacteria (Gupta et al., 2004).

Table 1 has also indicated that natural dyes had significant antibacterial activities towards the gram-positive bacteria and had less inhibition effect on the gram-negative test bacteria (*S. sonnei, E. coli, P. aeruginosa* and *Salmonella* sp.). The cell wall structure of the gram-negative bacteria is constructed essentially with LPS that avoids the accumulation of the antimicrobial agents on the cell membrane (Rabe and Staden, 1997; Ali-Shtayeh et al., 1998; Bezić, et al., 2003). The above reports have confirmed our results.

Antimicrobial activity of natural dyes on substrate

It was important to study the antimicrobial activity on dyed textile substrate (wool fabric) because the natural dyes showed inhibition effect against test bacteria in solution. The results are shown in Table 2. A reduction of 4-80% in bacterial growth is seen on a wool sample dyed with *P. granatum* and a reduction of 53-86% on wool samples dyed with *A. cepa*. A reduction of 32-52% in bacterial growth is seen on a wool sample dyed with *R. tinctorum* and a reduction of 28-91% in bacterial growth is seen on a wool sample dyed with *Mentha* sp. Also maximum inhibition rates (respectivelly, 80, 86, 52%) were obtained against *B. subtilis* of wool samples dyed with *P. granatum*, *A. cepa*, *R. tinctorum* while maximum inhibition rates (91%) was found against *P. aeruginosa* of wool sample dyed with *R. tinctorum.*

Han and Yang (2005) also observed an inhibition rate of 70% against *S. aureus* when 0.01% of curcumin was applied to the fabric and also 70% inhibition rate against *E. coli* with 0.05% of curcumin. However, inhibition rate of more than 95% was obtained against both *S. aureus* and *E. coli* when 0.2% of curcumin was applied to the fabric (Han and Yang, 2005). The results from these experiments indicated that these natural dyes had antimicrobial

Table 1. The antimicrobial activity of natural dyes against test bacteria (inhibition zone, mm).

Test bacteria	Dye			
	P. granatum	*R. tinctorum*	*M. piperita*	*A. cepa*
B. cereus RSKK 863	17.9 ± 0.0	-	10.8 ± 0.6	10.0 ± 0.0
B. megaterium RSKK 5117	12.4 ± 0.1	8.5±0	11.8 ± 0.2	13.5 ± 0.0
Shigella sonnei RSKK 877	13.8 ± 0.1	-	-	-
S. aureus ATCC 25923	13.7 ± 0.3	9.7±0	-	-
B. subtilis RSKK 244	17.6 ± 1.7	10.2±0	13.5 ± 1.6	9.9 ± 0.0
P. aeruginosa ATCC 29212	15.8 ± 2.0	-	12.2 ± 0.6	-
Salmonella sp. 21.3	10.5 ± 1.5	-	-	-
P. aeruginosa ATCC 27853	11.6 ± 0.5	-	10.2 ± 0.9	-
S. epidermidis	-	-	10.7 ± 0.6	-

Table 2. Antimicrobial activity (% reduction) of textile materials dyed with natural dyes.

Test bacteria	Dye			
	P. granatum	*A. cepa*	*R. tinctorum*	*M. piperita*
B. cereus RSKK 863	61	53	—	75
B. megaterium RSKK 5117	35	—	—	—
Shigella sonnei RSKK 877	24	—	—	—
S. aureus ATCC 25923	4	—	32	—
B. subtilis RSKK 244	80	86	52	88
P. aeruginosa ATCC 29212	79	—	—	91
Salmonella sp. 21.3	—	—	—	—
P. aeruginosa ATCC 27853	12	—	—	46
S. epidermidis	—	—	—	28
E. coli ATCC 35218	—	—	—	—

activity both on solutions and substrate. However, further research is needed to determine the effect of dye structure on inhibition.

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