

Antimicrobial activity of some natural dyes

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Received 1 April 2004; received in revised form 20 June 2004; accepted 10 September 2004

Available online 11 November 2004

Abstract

The present study was taken up as an exploratory study to test if some natural dyes have inherent antimicrobial activity with a view to develop protective clothing from these. Four natural dyes *Acacia catechu*, *Kerria lacca*, *Quercus infectoria*, *Rubia cordifolia* and *Rumex maritimus* were tested against common pathogens *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. *Quercus infectoria* dye was most effective and showed maximum zone of inhibition thereby indicating best antimicrobial activity against all the microbes tested. Minimum inhibitory concentration was found to be varying from 5 to 40 µg. The textile material impregnated with these natural dyes, however, showed less antimicrobial activity, as uptake of these dyes in textile material is below MIC.

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Keywords: Antimicrobial activity; Textile; Natural dye

1. Introduction

Textile materials and clothing are known to be susceptible to microbial attack, as these provide large surface area and absorb moisture required for microbial growth [1]. Natural fibres have protein (keratin) and cellulose, etc., which provide basic requirements such as moisture, oxygen, nutrients and temperature for bacterial growth and multiplication. This often leads to objectionable odour, dermal infection, product deterioration, allergic responses and other related diseases [2]. This necessitates the development of clothing that could provide a desired antimicrobial effect. A variety of antimicrobial textile materials are reported, employing different approaches like development of antibacterial nylon fibre by attaching a phosphate glass as an antibacterial agent [3]; surface coating by trialkoxysilyl

quarternary ammonium salt, antibacterial fibre by graft polymerization of cellulosic fibre with polyvinyl pyrrolidone, by treatment with potassium iodide solution; microencapsulation or insolubilization of chemical reagents in/on the fibre such as nitro compounds: 5-nitrofurfural, 5-nitro-2-furfurilidene-3-amino-2-oxazolidone, etc., on acrylic, nylon, cellulose, polypropylene and polyethylene fibre [4]. These recent approaches use synthetic non-biodegradable chemical compounds, which cause environmental and health concern.

Although known for a long time for dyeing as well as medicinal properties, the structures and protective properties of natural dyes have been recognized only in the recent past. Many of the plants used for dye extraction are classified as medicinal, and some of these have recently been shown to possess remarkable antimicrobial activity [5]. *Punica granatum* and many other common natural dyes are reported as potent antimicrobial agents owing to the presence of a large amount of tannins. Several other sources of plant dyes rich in naphthoquinones such as lawsone from henna,

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juglone from walnut and lapachol from alkannet are reported to exhibit antibacterial and antifungal activity [6–8].

However, dyes commonly used in textile are seldom screened for use as antimicrobial agents for textile finishing. The present study was undertaken to determine bactericidal properties of five commercially available dye powders, namely *Acacia catechu*, *Kerria lacca*, *Quercus infectoria*, *Rubia cordifolia* and *Rumex maritimus*, against some common microbes. Most of the studies conducted earlier have reported the activity of plant materials against *Candida rugosa*, *Staphylococcus aureus* and other drug resistant bacteria [9]. No study has been conducted so far to check the antimicrobial activity of these compounds when applied on textile materials.

For the current study, common pathogenic bacteria *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* have been selected as per the ASTM specifications. Human beings are commonly exposed to these microbes in household or hospital environments. These textiles dyed with these natural dyes can be very useful in developing clothing for infants, elderly and infirm people to protect them against common infections. They will be equally useful in bed linen, carpets and other home textiles, which are major propagators of common infections.

2. Experimental

2.1. Natural dyes

The optimized natural dye powders of *Acacia catechu*, *Kerria lacca*, *Quercus infectoria*, *Rubia cordifolia* and *Rumex maritimus* were obtained from ALPS Industries Ltd, Ghaziabad, India.

2.2. Wool fabric

Wool fabric having 2/2 twill weaves, 22 ends and 38 picks thread per inch, 163.6 warp and 59.1 weft yarn tex, 232 gsm (gram per square metre), 1.13 mm thickness and 81.4 cover factor was used for this study.

Wool was dyed by the standard method prescribed for natural dyes [10]. The dyeing was carried out at 10% owf (on weight of fabric), at 1:30 MLR (material to liquor ratio), for 30 min at 80 °C at neutral pH. Dyed samples were further treated with the non-ionic detergent Lissapol N (0.5 g/L) at 60 °C for 20 min, and rinsed in hot and then cold water.

2.3. Test organisms

Cultures of following microorganisms were used in the study: *Escherichia coli*, *Bacillus subtilis*, *Klebsiella*

pneumoniae, *Proteus vulgaris* and *Pseudomonas aeruginosa*.

2.4. Antimicrobial screening test

Nutrient agar medium (g/L: peptone 5.0; beef extract 1.5; yeast extract 1.5; NaCl 5.0; agar 20; pH 7.5) was prepared and autoclaved at 121 °C for 20 min. Sterilized petriplates were prepared with an equal thickness of nutrient agar. Test organisms were grown overnight at 37 °C, 120 rpm in 10 mL nutrient broth. This broth was used for seeding the agar plates. 10 µg of each dye was impregnated onto a small disc of filter paper (diameter 5.0 mm) and placed on top of the seeded medium. After overnight incubation at 37 °C, the zones of inhibition were measured.

In the second set of experiments, concentration of dye impregnated (5–40 µg) onto a disc of filter paper was varied to study its effect on the growth of microbes and MIC of dye.

The antimicrobial efficacy of a compound will vary when it is present in solution and when it is held intimately by a textile substrate. In the next set of experiments the antimicrobial activity of dyed wool specimens was tested. The 1 inch² fabric (dyed and undyed) was introduced in the 100 mL nutrient broth inoculated with the desired microbe and incubated at 37 °C overnight (16 h). The reduction of bacterial growth by dye was expressed as follows:

$$R = B - A / A \times 100$$

where R = % reduction in bacterial population; B = absorbance (660 nm) of the media inoculated with microbe and undyed fabric; A = absorbance (660 nm) of the media inoculated with microbe and dyed fabric.

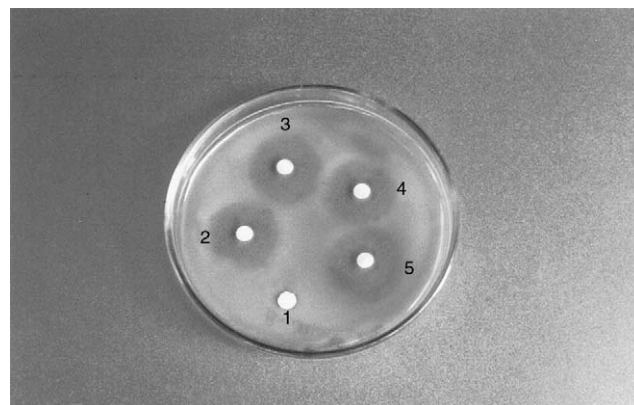


Fig. 1. Antimicrobial activity of the dye *Quercus infectoria* on *Bacillus subtilis*: Agar diffusion test for the effect of *Quercus infectoria* against *Bacillus subtilis* grown on nutrient agar medium. 1, control; 2, dye (5 µg); 3, dye (10 µg); 4, dye (20 µg); 5, dye (40 µg).

3. Results and discussion

3.1. Antimicrobial activity of natural dyes in solution

Natural dyes were screened for their anti-microbial activity against selected microbes (*Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*). The preliminary screening showed that *Quercus infectoria* was effective against all the microbes. A clear zone of inhibition by *Quercus infectoria* for *Bacillus subtilis* can be seen as a typical example in Fig. 1; *Acacia catechu* was effective against all microbes tested except *Pseudomonas aeruginosa*. *Rubia cordifolia* and *Rumex maritimus* were effective against *Klebsiella pneumoniae*. *Kerria lacca* showed no activity against any of selected microbes.

The effect of concentration of dye on antimicrobial activity was studied further and results are summarized in Table 1. The zone of inhibition (diameter) was recorded in each case. It was observed that increase in dye concentration leads to increased inhibition reflected by enhancement in diameter. It may be concluded that the dyes are highly effective antimicrobial agents as the MIC for most of these lies in region of 5–40 µg. *Rumex maritimus* was active only against *Klebsiella pneumoniae* and shows a small zone of inhibition. *Rubia cordifolia* showed little activity against *Klebsiella pneumoniae* at highest test concentration (40 µg). It is evident from Table 1 that with increasing concentrations of dye, the zone of inhibition is increasing almost linearly. The increase is much larger for *Quercus infectoria* than *Acacia catechu*. From the clear zone of inhibition obtained, it is apparent that the selected dyes are bactericidal in nature and not bacteriostatic.

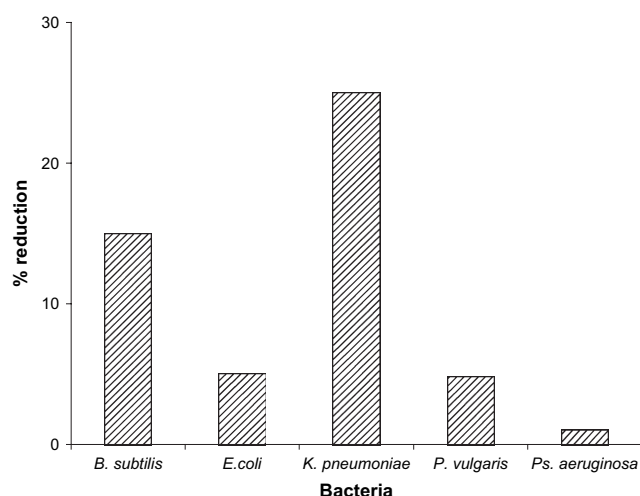


Fig. 2. Antimicrobial activity of textile materials dyed with *Quercus infectoria*: One square inch of sterilized fabric was introduced in 100 mL sterilized nutrient broth. This was aseptically inoculated with the microbe and incubated at 37 °C for 16 h. A broth without dyed fabric but similarly inoculated was kept as control. The absorbance of test and control was recorded at 660 nm. The antimicrobial activity was calculated as reduction in bacterial growth in sample compared to control using the formula described in Section 2.

3.2. Antimicrobial activity of natural dyes on substrate

Since dyes showed good antimicrobial activity against selected microbes in solution, it was thought worthwhile to study their antimicrobial activity on dyed substrate (wool). The wool samples dyed with these natural dyes were used as model system. The results are shown in Figs. 2 and 3. A reduction of 10–15% in bacterial growth is seen on a wool sample dyed with

Table 1
Zone of inhibition for natural dyes against selected microbes

Dye	Conc. (µg)	Zone of inhibition (diameter in cm)				
		<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Proteus vulgaris</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>
<i>Quercus infectoria</i>	5	0.9	2.5	1.2	2.0	0.7
	10	1.3	2.6	1.5	2.2	1.4
	20	1.6	2.7	1.7	2.4	1.9
	40	1.8	2.9	2.0	2.6	2.1
<i>Acacia catechu</i>	5	—	—	0.8	1.2	0.5
	10	—	0.6	0.9	1.4	0.8
	20	—	1.1	1.2	1.5	1.3
	40	—	1.3	1.3	1.6	1.5
<i>Rumex maritimus</i>	5	—	—	—	0.9	—
	10	—	—	—	0.9	—
	20	—	—	—	1.1	—
	40	—	—	—	1.2	—
<i>Rubia cordifolia</i>	5	—	—	—	—	—
	10	—	—	—	—	—
	20	—	—	—	—	—
	40	—	—	—	0.7	—

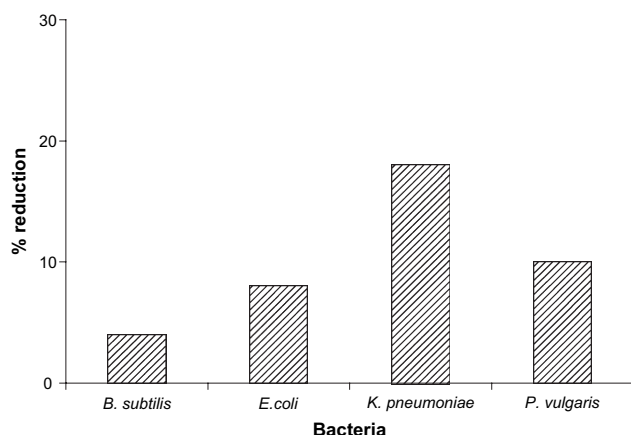


Fig. 3. Antimicrobial activity of textile materials dyed with *Acacia catechu*. The experiment was performed as described in the legend to Fig. 2.

Acacia catechu and a reduction of 15–25% on wool samples dyed with *Quercus infectoria*. An interesting observation is that *Quercus infectoria*, which exhibit high bactericidal activity in solution found to be bacteriostatic when dyed onto wool fibre. This may be because the concentration of dye (5.3% owf) on fabric samples is not sufficient enough for bactericidal activity. Even a 9.2% owf *Acacia catechu* dye is not sufficiently effective. Although both the dyes are tannin based, their anti-microbial activity differs greatly. This is an interesting finding and requires more in-depth investigation into the effect of dye structure on antimicrobial property. It is obvious that antimicrobial properties are

closely related to the dye structure, especially the presence of functional groups on it.

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